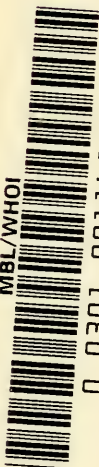


MBL/WHOI



0 0301 0011680 2



Seymour S. Cohen

CELL HEREDITY

RUTH SAGER, Research Associate in Zoology
FRANCIS J. RYAN, Professor of Zoology
Columbia University

New York · London, John Wiley & Sons, Inc.

431
521
C.2

1984

Gift of S. Cohen

CELL HEREDITY

SECOND PRINTING, MAY, 1962

Copyright © 1961 by John Wiley & Sons, Inc.

All Rights Reserved. This book or any part thereof must not be reproduced in any form without the written permission of the publisher.

Library of Congress Catalog Card Number: 61-11498

Printed in the United States of America

Preface

Genetics is the core science of biology. Its ultimate subject matter is a class of chemical compounds, the hereditary determinants, which are the prime movers of cellular metabolism and, thereby, of the even more complex processes of development and evolution.

Historically, genetics began with the demonstration of genes which behaved as unit factors in sexual transmission in higher plants and animals. Subsequently, in a great burst of fruitful research, genetics became established as a predictive science dealing with the organization and behavior of hereditary material at the biological level. Genetics is unique in biology for its broad and fundamental theory, and for methods which provide approximate solutions of the problems posed. In the 1930's it was already possible to build upon the theory of genetics, for example, in the experimental study of evolution, in the analysis of genetic effects upon physiology and development, and in application to plant and animal breeding.

Today, the science of genetics is coming to grips with the analysis of heredity at the molecular level in studies of the hereditary determinants: their chemistry, the molecular basis of their replication, mutation, and transmission, and the translation systems by means of which they exert their control over all cellular processes.

Progress in molecular genetics began to accelerate following the introduction of microorganisms into genetic research, a development which has been gaining momentum steadily since about 1940. In the last 20 years, there have been tremendous advances in this field, and in its impact upon other areas of biology. With microorganisms it has been possible to perform certain decisive experiments, technically infeasible with higher organisms, which have clarified and solidified points of genetic theory previously the subject of speculation. At the same time, some wholly un-

expected new findings have emerged which are forcing a revaluation and broadening of concepts in the entire science.

It has seemed timely, therefore, to attempt a summary statement, in the form of a modern textbook, of what appear to us the essential features of our science, and the principal directions of current inquiry. Such a statement must be understood to be tentative, with many areas of uncertainty, as befits a science in a fluid state. When they seemed important, we have included concepts about which there is considerable doubt, always indicating where this is the case. After all, the testing of concepts, and not their certainty, is the heart of science. The basic tenets of genetics are presented in terms of key illustrative experiments, and we have tried to capture the critical mood of the scientist and the controversial aspects of the findings as well as the facts.

In this book we have abandoned the chronological approach and have attempted a synthetic presentation, organizing the knowledge in a logically sequential pattern wherever possible. Thus we begin not with Mendel's pea crosses of 1866, but with the transformation experiment of 1943, which provided the first direct evidence of the chemical composition of hereditary material. In all fields of cell biology, the direction of progress has been towards a more mechanistic analysis of events on a physico-chemical basis. In genetics, the largest stride in this direction occurred with the chemical identification of nucleic acids as carriers of hereditary information. Nucleic acids may not be the sole hereditary determinants in the cell, but at least they represent one class, the only class yet detected; as such they provide the best frame of reference for a review of the facts and fancies of genetics, as they appear in 1960.

Although this is not a textbook of microbial genetics, experiments with microorganisms are heavily cited because they have provided material for many incisive experiments. We have limited ourselves to the discussion of genetics at the cellular level, and have omitted the application of genetic principles to scientific agriculture, to the experimental analysis of evolution, and to related fields. Although in the past, cell genetics and population genetics have been presented side by side, these fields have become so divergent in methodology and frame of reference that it seems preferable to treat them independently.

Genetics lends itself well to a formal presentation, largely abstracted from descriptive details and, therefore, it can be understood by persons who lack a background in biology. Until recently, even a lack of knowledge of physics and chemistry was no barrier, but as new experiments explore the molecular behavior of hereditary determinants, an elementary knowledge in the physical sciences becomes essential.

This book is addressed to the curious-minded of all ages from college

students to mature scholars in disciplines other than genetics. It is written out of an awareness that genetics is rapidly becoming of keener interest to scientists in other fields, as the impact of genetic determinants is felt in their experiments, and to students in search of challenging research problems. It is written especially in the hope that for some readers the acquisition of a broader understanding about a particular branch of science may be an exhilarating intellectual experience.

In preparing this book, we were aided by many friends and colleagues who provided us generously with information, illustrations, and criticism. We wish to acknowledge our deep appreciation to the following persons:

C. B. Anfinsen, R. Appleyard, K. C. Atwood, H. Crouse, M. Demerec, M. Fischberg, S. Fogel, P. E. Hartman, N. H. Horowitz, R. D. Hotchkiss, J. Iino, F. Gros, K. Kimura, J. Lederberg, E. B. Lewis, O. Maaloe, B. McClintock, G. E. Palade, D. D. Perkins, M. M. Rhoades, F. Stahl, J. H. Taylor, M. Tsujita, H. Vogel, M. Westergaard. We are particularly indebted to S. Fogel, B. N. Jamin, and D. D. Perkins for their detailed criticisms of the entire manuscript, and to G. E. Palade for the electron micrographs.

New York,
April 1961

RUTH SAGER
FRANCIS J. RYAN

Contents

1.	The chemical basis of heredity	1
	<i>Identification of Hereditary Material</i>	3
	<i>Genes and Gene Action</i>	28
2.	The mutable unit of heredity	34
	<i>The Particulate Nature of the Genetic Unit</i>	35
	<i>The Reversibility of Mutation</i>	39
	<i>An Operational Definition of the Mutable Unit</i>	40
	<i>The Randomness of Natural Mutation</i>	43
	<i>Mutation Rates</i>	53
	<i>Conclusion</i>	57
3.	Recombination in sexual organisms	60
	<i>Segregation and Assortment</i>	60
	<i>Linkage, Recombination, and Genetic Maps</i>	64
	<i>Genes on Chromosomes</i>	66
	<i>Crossing over and Chromosome Mapping</i>	73
	<i>Limitations in Mapping</i>	78
	<i>Transmission Genetics of Diploids</i>	83
4.	Cytogenetic correlations and crossing over	91
	<i>Chromosomes as Seen in the Light Microscope</i>	93
	<i>Chromosome Breakage and Rearrangements</i>	96
	<i>Variations in Chromosome Number</i>	104
	<i>Crossing Over</i>	106
5.	Recombination in viruses and bacteria	113
	<i>Mating among Viruses</i>	114

	<i>Lysogenic Bacteria and Prophage</i>	122	
	<i>Transduction of Bacterial Genes via Bacteriophage</i>	125	
	<i>Transformation by DNA</i>	131	
	<i>Mating in Bacteria</i>	134	
6.	What is a gene?		150
	<i>Position Pseudoallelism</i>	153	
	<i>The Structure of Genes in Salmonella</i>	158	
	<i>The Structure of the Gene in Bacteriophage</i>	163	
	<i>A Concept of the Gene</i>	167	
7.	Chromosome duplication and genetic recombination		175
	<i>Comparative Organization of Genetic Material</i>	176	
	<i>Chemical Composition of Chromosomes</i>	178	
	<i>Electron Microscope Studies of Chromosomes</i>	180	
	<i>Integration of DNA in the Bacterial Cell</i>	183	
	<i>DNA and Chromosome Duplication</i>	184	
	<i>Recombination Revisited</i>	195	
8.	Mutation as a chemical process		207
	<i>Mutation and Gene Replication</i>	207	
	<i>Incorporation of Unusual Nucleotides and Copy Errors</i>	212	
	<i>Point Mutations Versus Grosser Changes in the Gene</i>	217	
	<i>Mutations Induced by Radiation</i>	219	
	<i>The Metabolic Control of Mutation</i>	225	
	<i>Directed Mutation</i>	231	
9.	Nonchromosomal genes		234
	<i>Definitions and Criteria</i>	235	
	<i>A Review of Cell Structure</i>	244	
	<i>Chloroplast Heredity</i>	249	
	<i>Mitochondrial Heredity</i>	254	
	<i>Nonchromosomal Heredity Involving No Known Structures</i>	257	
	<i>Infectious agents</i>	262	
	<i>Concluding Remarks</i>	268	
10.	Some mechanisms of gene action		271
	<i>The Search for Analyzable Traits</i>	271	
	<i>Genetic Block of Single-Step Reactions</i>	273	
	<i>Qualitatively Altered Enzymes</i>	274	
	<i>Tryptophan Synthetase</i>	276	
	<i>Hemoglobin</i>	280	
	<i>Some Concepts of Protein Structure</i>	283	

	<i>Allelic Complementation</i>	291	
	<i>Suppression</i>	297	
	<i>DNA Base Ratios and Gene Action</i>	299	
	<i>Resumé</i>	300	
11.	Genetic control of cell integration		305
	<i>Protein Synthesis</i>	308	
	<i>Nucleic Acid Synthesis</i>	311	
	<i>Genetic Control of Enzyme Formation</i>	321	
	<i>Mutations Affecting Rates of Enzyme Formation</i>	334	
	<i>Position Effect and Controlling Elements</i>	338	
	<i>Nuclear Transfer</i>	351	
	<i>Concluding Remarks</i>	352	
12.	Heredity in somatic cells		356
	<i>Nuclear Differentiation</i>	356	
	<i>Recombination in Somatic Cells</i>	359	
	<i>Somatic Mutation and Selection</i>	370	
	<i>Infectious Heredity</i>	378	
	<i>Phenocopies</i>	380	
	<i>Cell Population Dynamics</i>	382	
13.	The summing up		386
	<i>Nature of Hereditary Materials</i>	386	
	<i>Transmission Mechanisms</i>	388	
	<i>Gene Action</i>	389	
	<i>Replication and the Origin of Life</i>	392	
	Postscript		397
	Author Index		403
	Subject Index		407

1 The chemical basis of heredity

Heredity is that process which brings about the biological similarity between parents and progeny. Genetics is the science of heredity. Its subject matter is the multitude of events which determines the reappearance of parental traits among progeny.

These events can be grouped into three categories dealing, first, with the nature of hereditary materials; second, with their replication and transmission from generation to generation; and third, with the translation systems by means of which the hereditary materials exert control over metabolism and development. These three categories are closely interrelated, but to some extent they can be considered independently. In this book Chapters 1, 2, 8, and 9 deal primarily with the identification of hereditary determinants; Chapters 3–7 with replication and transmission; and Chapters 10 and 11 with translation; in Chapters 12 and 13 some problems are discussed in which these categories are merged.

Historically, the methodology of genetics has been based not on the analysis of parent-progeny similarities but rather on *differences*, and to a considerable extent this is still the rule. The method consists essentially of mating unlike parents and analyzing the patterns of segregation of parental differences among the offspring. Some parental traits are not inherited at all, some give very complex patterns of segregation, but some segregate in a remarkably clear-cut manner. It was the recognition of this latter class which led to the explosively rapid development of genetics.

When two parents differ by a number of clear-cut traits, all progeny will be readily classifiable and will show regular patterns of segregation of each of these traits. When segregations are regular, predictions can be made about the outcome of future matings, and the method can be

expanded to handle more complex patterns of inheritance. From information obtained in this way, geneticists have identified a special class of cell constituents—the hereditary determinants, or *genes*. Genes are the units of inheritance with whose properties the science of genetics is concerned.

Genes have not been directly observed as yet, although recent experiments to be described in this book are bringing this possibility closer and closer to realization. However, the science of genetics was built by inference and prediction about genes from experiments in which the unit of observation was not the gene itself but its effect upon the organism. Two important terms were designed to keep clear the distinction between the postulated genes and their observed effects. As first proposed by the Danish geneticist Johanssen in 1911, the *genotype* is the sum total of the hereditary determinants or genes present in an organism and the *phenotype* is the sum total of observed characteristics of the organism. It is the phenotype which is observed in breeding experiments, and inferences about genes are made from the segregation of phenotypic differences.

The phenotype is influenced by all the hereditary determinants and by their interactions with environment as well. Consequently, the detection of single phenotypic differences resulting from single gene differences can be very difficult, and the choice of favorable experimental material is of key importance. The science of genetics began with studies of complex organisms such as the pea, the fruitfly, the corn plant, and the house mouse. Observations were directed at traits—taillessness in mice for example—far removed from the intracellular level where the genes are located and where they exert their primary effects. The creation of a powerful analytical science by inference from the results of these highly indirect experiments represents a great intellectual achievement of the pioneer geneticists. However, both the achievements and the limitations of classical genetics derive from the same source: the remoteness of the experimental observations from the primary actions of the hereditary materials themselves.

In an attempt to bridge this gap, some investigators began to employ single-celled organisms such as bacteria, yeast, algae, fungi, and protozoa. By avoiding the complexities of tissue differentiation and integration, they were able to concentrate fully upon events at the cellular level. In these systems the parent-progeny relation may be either sexual, involving offspring derived from mating of two parents, or asexual, involving daughter cells produced by fission of a single parental cell.

The cellular approach has made possible a great refinement in concept and in method, so that more precise questions can be asked and more definitive experiments performed. The direction of advance has been

toward description on a molecular level, toward a science in which the events of heredity can be described in terms of physics and chemistry, and toward a physical concept of the gene that will allow prediction of its behavior.

It should be emphasized, however, that genetics cannot yet be presented as a molecular science. It is a science in transition, from the purely biological to the physicochemical level of analysis, with some areas more advanced in this direction than others. Our choice of illustrative material has been weighted heavily toward experiments interpretable on a physicochemical basis. Frequently these are very recent experiments. Consequently, we have been led to consider current problems in genetics much more extensively than past achievements. In this sense, this book is an introduction to research areas in cellular genetics.

IDENTIFICATION OF HEREDITARY MATERIAL

The single most illuminating and significant advance in modern genetics to date has been the chemical identification of a class of macromolecules—the nucleic acids—as hereditary determinants. This finding has ramifications and implications which we shall be considering in various contexts throughout the book. One important consequence has been the opportunity of experimenting directly with hereditary materials instead of relying solely upon inferences from indirect observation.

In this chapter we shall examine the experimental evidence that hereditary determinants have indeed been identified; and we shall discuss some of their properties.

Formal methodology

Consider two bacterial cells which differ by a single observable characteristic: one is colored (a^+) and the other is colorless (a^-) when grown in the same constant environment. Each cell grows and then divides into two daughter cells which, in turn, repeat the process and, after n divisions, there are 2^n progeny, which arose from a single parental cell; together they form a *clone*. On inspection, one finds further that all cells of the a^+ clone (which came from the a^+ parental cell) are a^+ , and all cells of the a^- clone are a^- . Thus the color characteristic is a hereditary property of the organism and is regularly transmitted to all progeny.

Suppose that one makes various chemical extracts of the a^+ clone. If one treats a^- cells with extracts from a^+ cells, can one transform a^- to a^+ ? Here we must distinguish carefully between (1) a physiological

change from a^- to a^+ which results from the influence of the extract on cellular metabolism and (2) a hereditary change from a^- to a^+ which persists indefinitely among all progeny of the treated cells in the absence of the extract because of the presence of a new hereditary determinant. Various compounds from a^+ cells may assist a^- cells to form pigment, and thereby to look like a^+ , but the progeny clones derived from such cells are again a^- . Such a^- cells are called *phenocopies*; they are altered physiologically, but remain genetically a^- . Here we are not concerned with physiological effects, but rather with the question: Is there any chemical constituent within the a^+ cell which can change the *heredity* of the a^- cell from a^- to a^+ ?

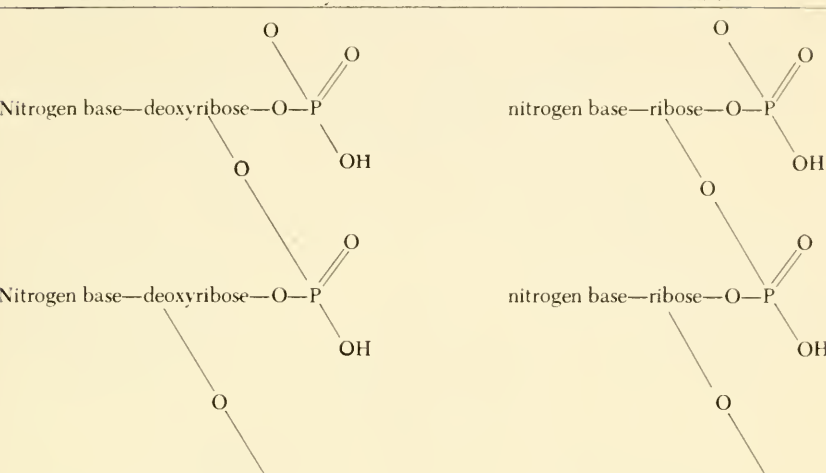
Suppose experiments could be performed successfully in which a particular molecular species extracted from one cell type, a^+ , the donor, could transform cells of a different cell type, a^- , the recipient, so that they became genetically a^+ and yielded upon extraction the same active compound. The transforming extract would then be acting either by altering a hereditary determinant of an a^- cell (mutation) or by introducing a new hereditary determinant from the a^+ clone. With more detailed information, one could distinguish between these alternatives. If the transforming extract were indeed introducing a new hereditary determinant into the a^- cell, then this new determinant would be present in the extract. Transformation experiments, therefore, provide a method for the isolation and identification of hereditary material.

Successful transformation experiments have been performed with several strains of bacteria, in which the symbols a^+ and a^- may be taken to represent any one of many pairs of different cell traits. Only one class of molecules has been found thus far with the ability to induce hereditary transformations. Analogous experiments have been carried out with a number of viruses and there too the same class of compounds has been found capable of transmitting viral properties. These compounds are *nucleic acids*.

Chemistry of the nucleic acids

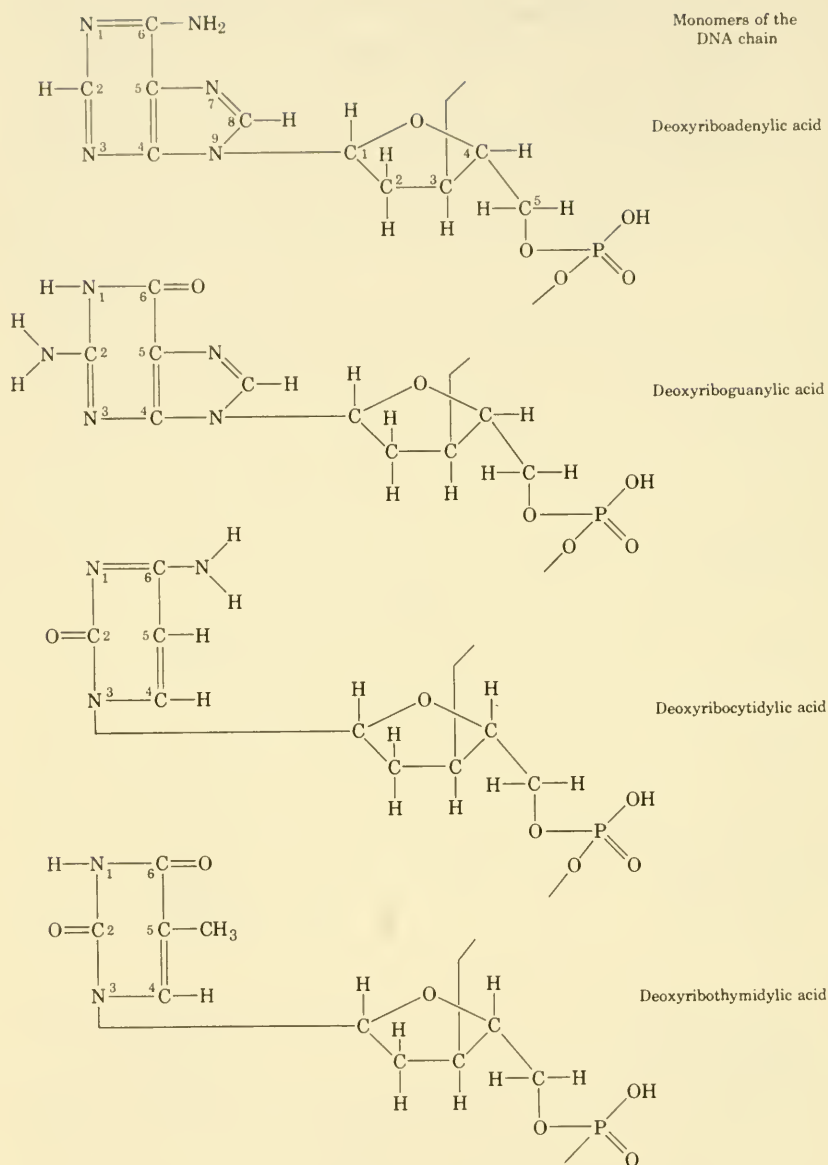
Nucleic acid molecules are long-chain polymers (polynucleotides) composed of monomeric units called *nucleotides*, which are themselves complex molecules, each consisting of three components: a nitrogen base, a sugar, and a phosphoric acid radical. A nucleotide without the phosphate is called a *nucleoside*. All nucleic acids so far studied fall into two classes: the DNA's (deoxyribonucleic acids) and the RNA's (ribonucleic acids), which differ from each other in the sugar moiety, in one of the nitrogen bases, and in many physical properties. The chemical relation-

TABLE 1.1
Chemical Relationships of DNA's and RNA'S

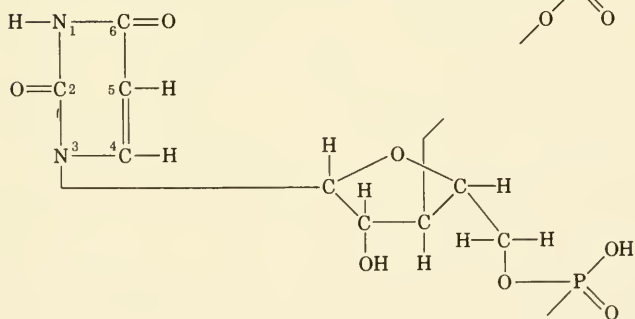
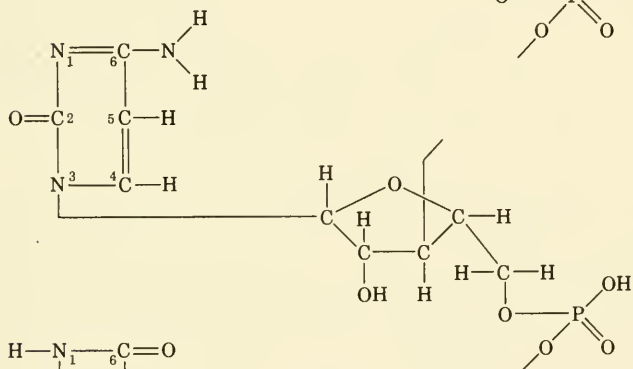
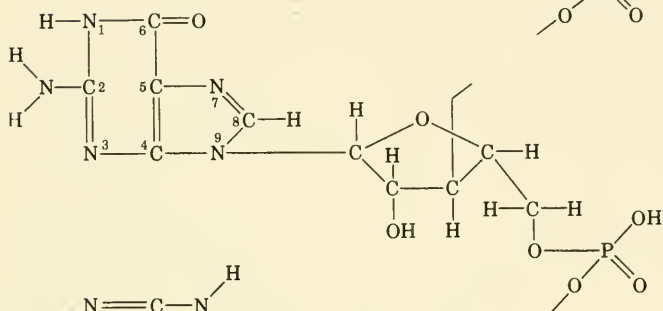
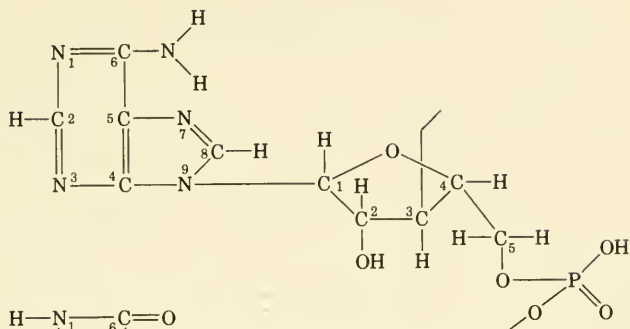
DNA	Polynucleotide Chains	RNA
		
Nitrogen bases purines Adenine Guanine pyrimidines Cytosine Thymine		
Nucleosides (nitrogen base + sugar) Deoxyadenosine Deoxyguanosine Deoxycytidine Deoxythymidine		adenine guanine cytosine uracil adenosine guanosine cytidine uridine
Nucleotides (nitrogen base + sugar + phosphoric acid) Deoxyriboadenylic acid Deoxyriboguanilyc acid Deoxyribocytidylic acid Deoxyribothymidylic acid		adenylic acid guanylic acid cytidylic acid uridylic acid

ships are summarized in Table 1.1 and structural formulae are given in Figure 1.1.

In addition to the eight common nucleotides listed here, a number of special constituents have been found in the nucleic acids of particular



1.1a. Structural formulae of the nucleotides of DNA.

Monomers of the
RNA chain

1.1b. Structural formulae of the principal nucleotides of RNA.

organisms. For example, in the DNA of certain bacterial viruses, all of the cytosine is replaced by 5-hydroxymethyl cytosine; in the DNA of some plants, 5-methyl cytosine replaces a part of the cytosine; and in certain cells, DNA is found to contain small amounts of other bases. With respect to sugars, only ribose (in RNA) and deoxyribose (in DNA) have been found, except for glucose which is present in addition to deoxyribose in the DNA of some bacterial viruses.

Each polynucleotide chain has a sugar-phosphate backbone, built of phosphodiester linkages which connect the phosphate of one nucleotide to the sugar of the next. The nitrogen bases project out from this backbone at angles of about 50° , differing slightly from base to base.

The precise linear order of nucleotides in the polynucleotide chain is a matter of great importance to the geneticist. If nucleic acids are hereditary material, they must be capable of a tremendous diversity of form in order to carry all the genetic information of the cell. The most apparent basis for such diversity between different nucleic acid molecules lies in the nucleotide sequence, which can be thought of as a code. If each of the four nucleotides of DNA, for instance, represents a different symbol, or letter of a four-letter alphabet, then words or messages can be composed on the basis of nucleotide sequence along the polynucleotide chain. At present, it seems very likely that nucleotide sequence provides a basis for individuality among the nucleic acid molecules of the cell. The available chemical evidence is consistent with this view.

Ideally, one would like to determine the actual linear order of bases in a nucleic acid molecule, but, if each cell contains a great variety of different types of molecules, the first problem is that of isolating one kind of molecule from the others. Until this feat is accomplished, total sequence analysis would be pointless. On the other hand, some indirect approaches to the question have been very revealing. Certain regularities in base composition have been established, as shown in Table 1.2. In all samples of DNA and RNA thus far analyzed (except the NAs of some viruses), the number of *6-keto* groups equals the number of *6-amino* groups. Adenine and cytosine each contain an amino group in the 6-position; and guanine, uracil, and thymine each contain a 6-keto group. The total number of adenines plus cytosines equals the total of guanines plus thymines in DNA, and of guanines plus uracils in RNA. There is a further regularity observed in DNA samples which is not found in most RNA's, namely, the molar ratio of adenine to thymine is one, and that of cytosine to guanine is one. Consequently, the sum of the purines equals the sum of the pyrimidines. However, the amount of adenine plus thymine relative to guanine plus cytosine varies considerably from species to species, as can be seen in Table 1.2.

TABLE 1.2
Base Ratios of some DNA's and RNA's

Organism	DNA				RNA			
	A/T	G/C	$\frac{A+T}{G+C}$	$\frac{6\text{-Amino}}{6\text{-Keto}}$	A/U	G/C	$\frac{A+U}{G+C}$	$\frac{6\text{-Amino}}{6\text{-Keto}}$
1. Tobacco mosaic virus					1.14	1.37	1.28	0.94
2. Phage T2	1.00	1.09	1.87	0.97				
3. Phage ϕ X174 (single-stranded DNA)	0.75	1.31	1.35	0.76				
4. <i>E. coli</i>	1.09	0.99	1.00	1.05	1.38	1.18	0.83	1.06
5. <i>Serratia marcescens</i>	1.03	0.85	0.69	1.15	0.84	1.28	0.80	0.81
6. Pneumococcus	0.945	1.14	1.59	0.92				
7. Yeast	0.964	1.08	1.80	0.95	0.93	1.087	1.12	0.927
8. Sea urchin	1.02	1.01	1.85	1.01	1.086	1.085	0.767	0.992
9. Starfish oocyte nucleoli					1.47	1.34	0.575	0.962
10. Salmon sperm	1.02	1.01	1.43	1.00				
11. Ox liver	0.99	1.00	1.37	1.00	0.79	0.80	0.63	1.04
12. Ox liver nuclei					0.805	0.865	0.795	0.990
13. Calf thymus	0.985	1.15	1.28	0.938				
14. Man—sperm	0.98	1.03	1.67	0.976				
15. Man—thymus	1.05	1.00	1.54	1.03				
16. Man—liver	1.00	1.00	1.54	1.00				

Certain conclusions can be drawn from these findings. In the first place, there is a constancy in base composition, such that repeated analyses of nucleic acids from particular organisms always give the same results. Secondly, the average base composition of the nucleic acids is species specific, differing from one organism to another. Thirdly, there seems to be more species variation in the DNA ratios than in the RNA ratios, but, since these values are averages of large numbers of different molecules, more than one interpretation is possible. These findings provide no evidence for or against the view that the total DNA and RNA extracted from the cell consists of a mixture of molecules, very heterogeneous in nucleotide sequence. As yet, most of the evidence in support of this view comes from genetic, not from chemical, experiments. We shall return to this question later.

Crystallographers have studied the three-dimensional configurations of DNA and of RNA molecules by means of X-ray diffraction, a method which reveals the regularities in atomic arrangement. The DNA preparations from many different organisms all show the same characteristic repeating pattern, which is best interpreted as resulting from the organ-

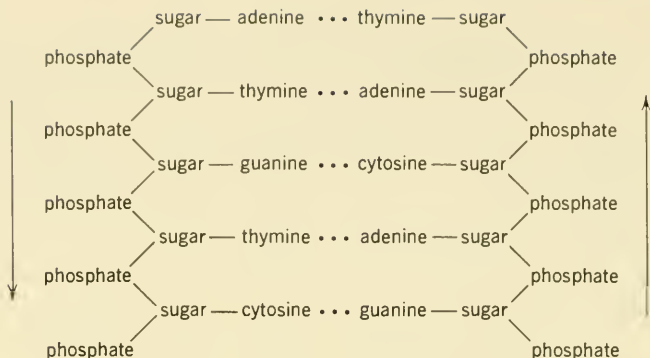


FIGURE 1.2a. Schematic diagram of DNA. The two chains are antiparallel, as shown by the arrows. The dotted lines between the bases represent hydrogen bonding. Although the chains are drawn flat in this diagram, they are actually wound around each other in the molecule, as in Figure 1.2b.

ization of polynucleotides into a double helix. This helix, shown in Figure 1.2, consists of two chains wound around a central axis, and held together by hydrogen bonds between nitrogen bases which are opposite each other, one on each chain.

By constructing scale models compatible with the X-ray data, Watson and Crick were able to show that the bases could fit into this double helix only if they were paired in a specific way—adenine with thymine and guanine with cytosine, as shown in Figure 1.3. This specific pairing requirement is entirely consistent with the base ratio data (Table 1.2); DNA molecules do contain precisely the molar ratios demanded by the double helical model. The excellent correspondence between chemical fact and crystallographic possibility has led towards acceptance of the double helix as the native configuration of DNA.

A most important consequence of the pairing relationship in the double helix is complementarity of the two polynucleotide strands. If the strands become separated, and a new strand is formed in relation to each of the old ones, then each new strand will be *identical* with the old strand with which it did not pair, and *complementary* with its partner. The sequence of nucleotides in the two strands will not be identical, but, because of their complementarity, either one could generate both in two cycles of replication.

Less information is available about the native configuration of RNA molecules, partly because, in contrast to DNA, they are not easily extracted from cells and purified without considerable structural damage. A current view is that RNA is a single-stranded polymer, not helical like

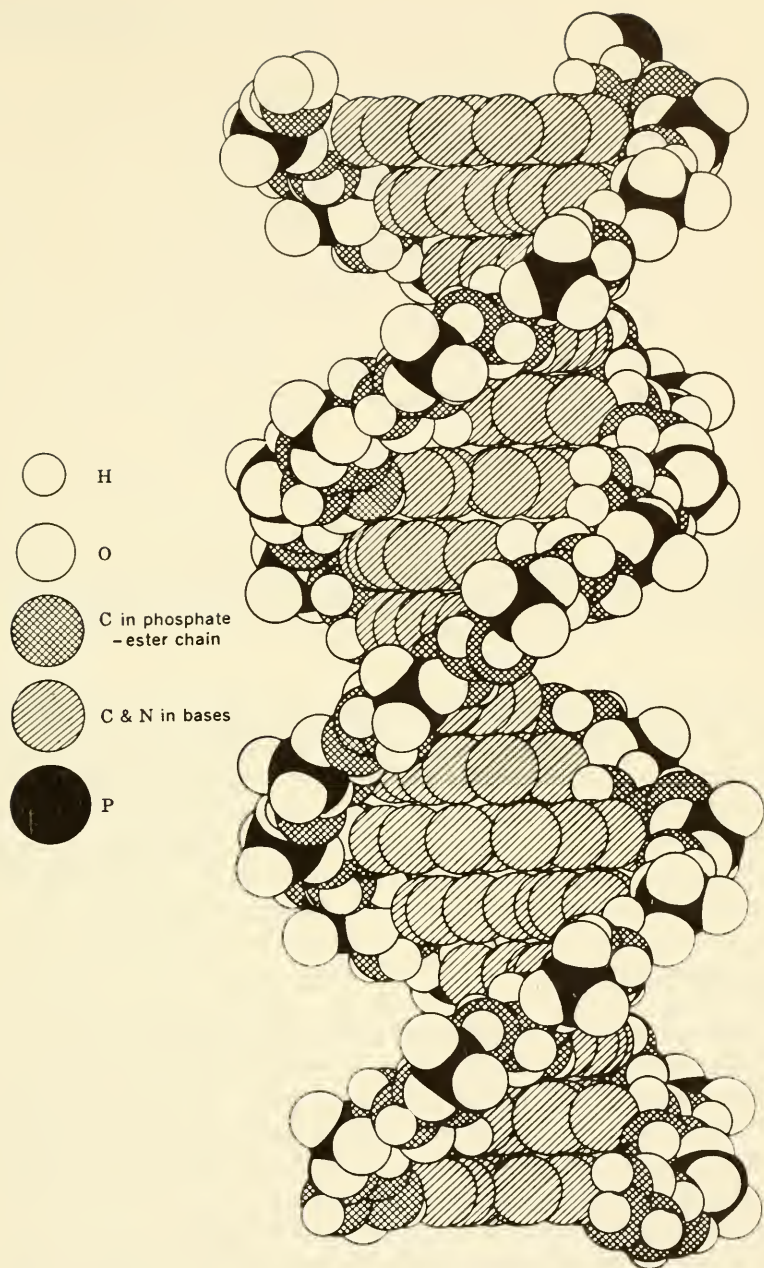


FIGURE 1.2b. Drawing of the DNA molecule in which solid circles illustrate atoms. There are two helical grooves of unequal size on the outside of the DNA molecule (after Feughelman, Langridge, Seeds, Stokes, Wilson, Hooper, Wilkins, Barclay, and Hamilton, 1955, *Nature*, 175:834).

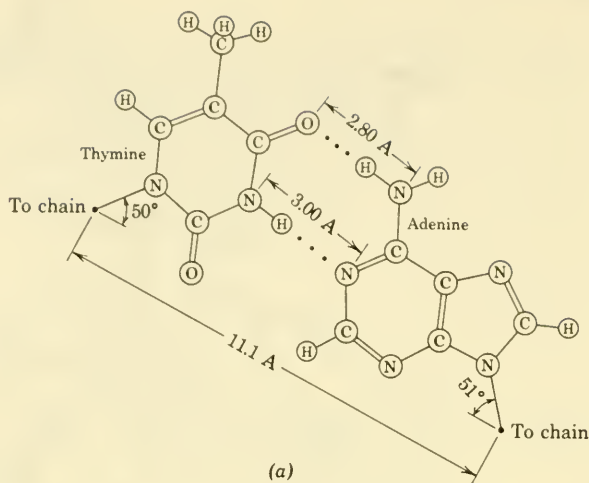


FIGURE 1.3a. Diagram showing the hydrogen bonding between adenine and thymine in DNA (from Pauling and Corey, 1956, *Arch. Biochem. Biophys.*, **65**:164).

DNA, but rather randomly arranged, with local regions held together by hydrogen bonds between the bases. The one regularity which has been found in RNA base ratios, namely, that the total of 6-keto-equals the total of 6-amino-containing bases, suggests the existence of some structural pattern which has not been identified as yet. The absence of other regularities in base ratio supports the view of RNA as a single- rather than a double-stranded molecule.

Bacterial Transformation

In 1928, in England, Griffith reported a most remarkable finding. In studying pneumonia, he had injected into mice a mixture of two strains of pneumococcus (*Diplococcus pneumoniae*); one strain was living but not virulent, inducing no infection, and the other strain was dead, heat-killed (although if it had been alive, it would have induced bacteremia). The mice died of bacteremia in this experiment, and from their bodies, living, virulent pneumococci of Type III were isolated.

Virulence in pneumococcus depends upon the presence of a polysaccharide capsule around the cells; many capsular types have been identified, differing in their detailed chemical composition. Cells are typed by immunological tests, which distinguish between subtle chemical differences in the complex polysaccharide molecules. Strains of each

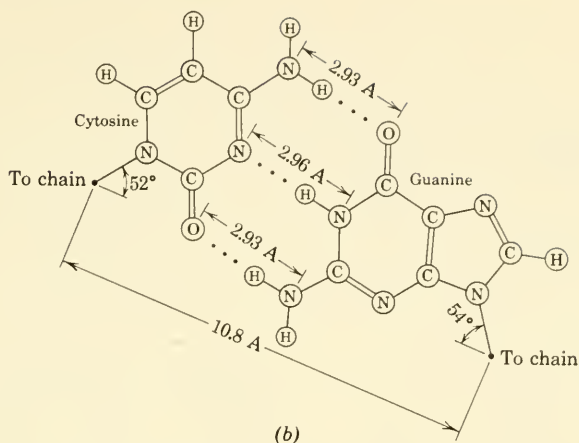


FIGURE 1.3b. Diagram showing the hydrogen bonding between guanine and cytosine in DNA. This pair is held together by three hydrogen bonds in contrast to the two found in the adenine-thymine pair (from Pauling and Corey, 1956, *Arch. Biochem. Biophys.*, **65**:164).

immunological type give rise only to progeny of the same type; type specificity is thus a hereditary property of the organism.

In Griffith's experiment the heat-killed virulent cells were of immunological Type III, and the nonvirulent strain was derived from Type II, as shown in Figure 1.4. No mice died in the control group, which received only one strain. These results suggest that some interaction occurred between the two strains by which the properties of virulence and type specificity were transferred from the heat-killed virulent strain of Type III to the living, nonvirulent strain, and furthermore, that a hereditary determinant was transferred, since all the progeny bacteria isolated from the dead mice remained heritably virulent and of Type III.

We can easily formulate this interpretation now, but it was only after intensive experimentation by several investigators that these results were understood. The work culminated in the classical papers of Avery and collaborators on transformation in pneumococcus.

Experimentation in Avery's laboratory was focused on: (1) repetition of Griffith's results with an *in vitro* system; and (2) isolation and chemical identification of the transforming substance responsible for converting avirulent cells into virulent ones, and one immunological type into another. These aims were achieved. Overcoming great technical difficulties, Avery and co-workers were able to demonstrate *in vitro* type transformation with a number of pairs of pneumococcal strains, although the

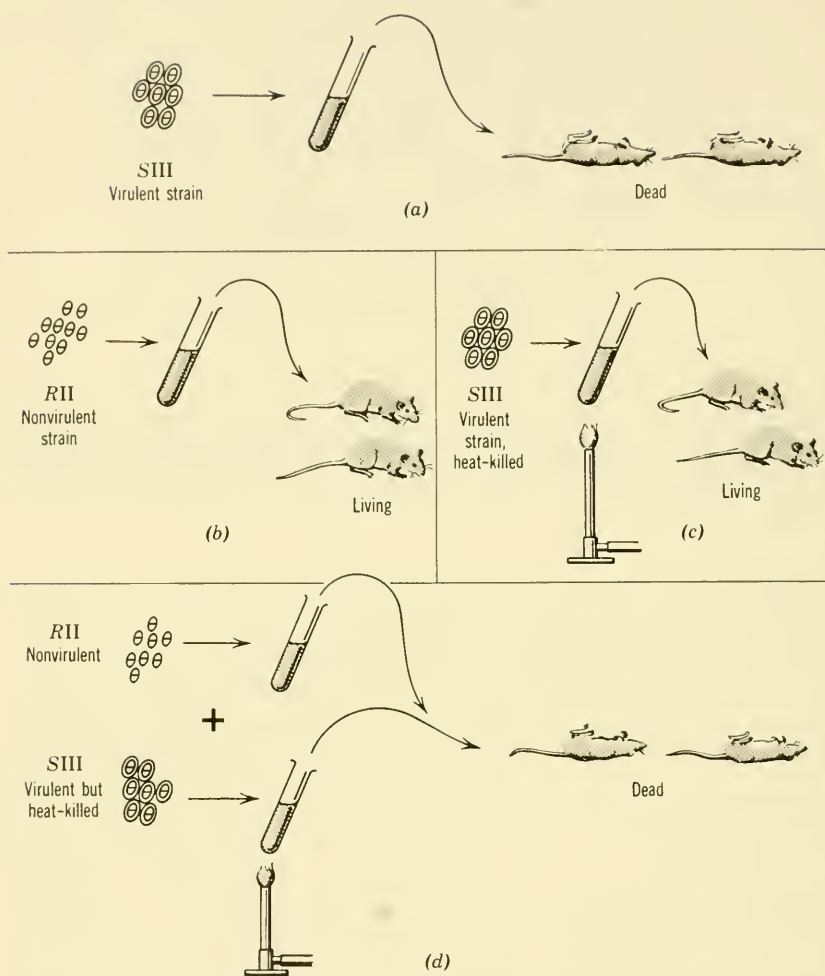


FIGURE 1.4. Diagram of Griffith's experiment.

- (a). Mice injected with a virulent strain of pneumococcus, SIII, die of bacteremia.
- (b). Mice do not die when injected with a nonvirulent strain, RII, which does not possess a capsule but comes from a virulent, encapsulated strain, SII.
- (c). Mice do not die when injected with the virulent strain, SIII, which has been heat-killed.
- (d). When mice are injected with a mixture of heat-killed SIII and nonvirulent RII, they die of bacteremia, and living virulent SIII organisms are found in their bodies.

frequency of transformation which they obtained was at first only about one per million cells treated.

A typical type transformation experiment is diagrammed in Figure 1.5. Nonencapsulated cells (derived from capsular Type II) are mixed with an extract from encapsulated cells of Type III, and the progeny which result are composed of two classes of cells: most of them are nonencapsulated, like the parental strain, but a few, about one per million, are encapsulated and of Type III, like the strain from which the extract was made. This result represents the *in vitro* confirmation of Griffith's original finding.

When the transformed cells were isolated and grown, not only did they remain encapsulated but extracts prepared from them had the same transforming ability as did extracts obtained from the original encapsulated strain. It was thereby demonstrated that *in vitro* transformation resulted in a hereditary change just as had occurred *in vivo*.

Using this experimental procedure as a biological assay for the transforming activity of various extracts from donor cells, Avery, McLeod, and McCarty were able to isolate and purify a single class of molecules, which they called transforming principle. They found that it consisted of deoxyribonucleic acid (DNA). Further purification of the extract and many analyses by other investigators have only confirmed and strengthened this conclusion. Although it cannot be shown that any extract is completely pure, there is no reason to believe that the activity of transforming extracts results from the presence of a trace contaminant. On the contrary, the behavior of the transforming extract coincides in

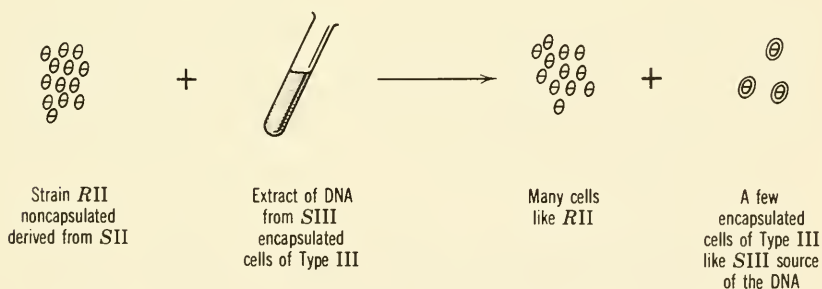


FIGURE 1.5. Transformation of capsular type in pneumococcus. Cells of strain RII, which are noncapsulated but derived from encapsulated strain SII are treated with an extract of DNA from encapsulated cells of strain SIII. After treatment, many cells are recovered like the parental strain RII but, in addition, some encapsulated cells of Type III are found like strain SIII which was the source of the DNA. These cells are *transformants*, having acquired from the DNA extract new genetic material for making Type III capsules.

every respect with the known properties of DNA. With every refinement of technique and new method of analysis, the identity of transforming principle as DNA has become more firmly established.

At the time, however, the finding that transforming principle was DNA came as a shock to its discoverers, who had rather expected that it would be the capsular polysaccharide itself, on the hypothesis of identity between the hereditary material and the observed trait. When polysaccharide could not be found in the active extract, it seemed reasonable that it might be a protein, for in 1944 only proteins were considered by most investigators to be sufficiently complex to determine cellular specificity and heredity. With the eventual identification of the transforming principle as DNA, based upon the most meticulous experimentation, Avery, McLeod, and McCarty established with great clarity two points of central importance in genetic theory: (1) the chemical *dissimilarity* between a hereditary determinant and the phenotypic trait which it determines; and (2) the ability of a molecule of deoxyribonucleic acid to carry hereditary information.

One may then ask whether transformation experiments exclude the possibility that DNA acts not as hereditary material but as a mutagenic agent which induces a change in the hereditary determinants of the receptor cell. This alternative has become steadily less probable with further studies of the transformation process. For example, with the use of DNA containing radioactive phosphorus, P^{32} , in transformation experiments, the actual incorporation of P^{32} -DNA into the receptor cells has been measured. It has been clearly shown in such experiments that the yield of transformed receptor cells closely parallels the extent of DNA incorporation.

Transformation has been achieved for a large number of different traits, but in all instances receptor cells are transformed only for the particular traits of the donor cells. The mutagen hypothesis would require that DNA be a highly specific mutagen corresponding precisely to the traits of the donor. In effect, then, the specific mutagen becomes operationally indistinguishable from a hereditary determinant.

Type transformation in pneumococcus thus provided the first system in which the chemical nature of a hereditary determinant was established. This first demonstration of the genetic significance of DNA was of epoch-making importance. The repercussions are only beginning to be felt in the recent acceleration of new findings in this field, which has appropriately been called genetic chemistry. On the one hand, the transformation experiment provides the chemist with the only available method for determining the biological intactness of the material that he extracts from the cell for analysis. Thus the biological assay is an

essential tool in the investigation of the physical chemistry of genetic material. On the other hand, the physical chemical studies are providing the biologist with an understanding of the molecular basis of the biological specificity and information-carrying capacity of the nucleic acids.

The experiments of classical genetics have provided evidence that hereditary information is carried in unit packets which have been called *genes*. The transformation experiments have provided evidence that genetic information is carried in nucleic acid molecules. What is the relation between the biological unit, the gene, and the chemical unit, the molecule? To answer this question, let us first consider evidence which identifies the biological unit by means of transformation experiments.

Unit factor transmission

The use of drug-resistant strains in transformation experiments provides a means for better quantification than has been possible with type specificity. Drug-resistant strains are found as rare variants in drug-sensitive populations. For example, when 10^9 penicillin-sensitive pneumococci are placed in contact with a killing concentration of penicillin on a solid medium, an occasional cell survives and forms a colony. If such a colony is then allowed to grow for many generations in the absence of penicillin, and its progeny are tested for resistance, each daughter cell will grow and form a colony on penicillin-agar, as shown in Figure 1.6. Thus the colony isolated from the first penicillin plate consisted of cells which differed from all the rest of the population in being penicillin-resistant. All of its progeny are resistant, and DNA extracts from such strains will transform sensitive cells into resistant ones. Similarly, DNA extracts from strains resistant to other drugs, such as streptomycin, cathemycin, and sulfanilamide, will transform sensitive cells into the corresponding resistant type. Doubly and triply resistant strains can be produced by serial steps of transformation, and one can test the ability of their DNA extracts to transform cells into each resistant type. Since

TABLE 1.3
Double Transformation with Unlinked Markers
(After Hotchkiss, 1954, Symposium on Genetic Recombination,
J. Cell. Comp. Physiol., **45**:1)

Donor DNA	Receptor Cells	Transformants	
		Single	Double
<i>pen-r str-r</i>	<i>pen-s str-s</i>	<i>pen-r str-s</i> 0.01	
			<i>pen-r str-r</i> 0.0001
		<i>pen-s str-r</i> 0.01	

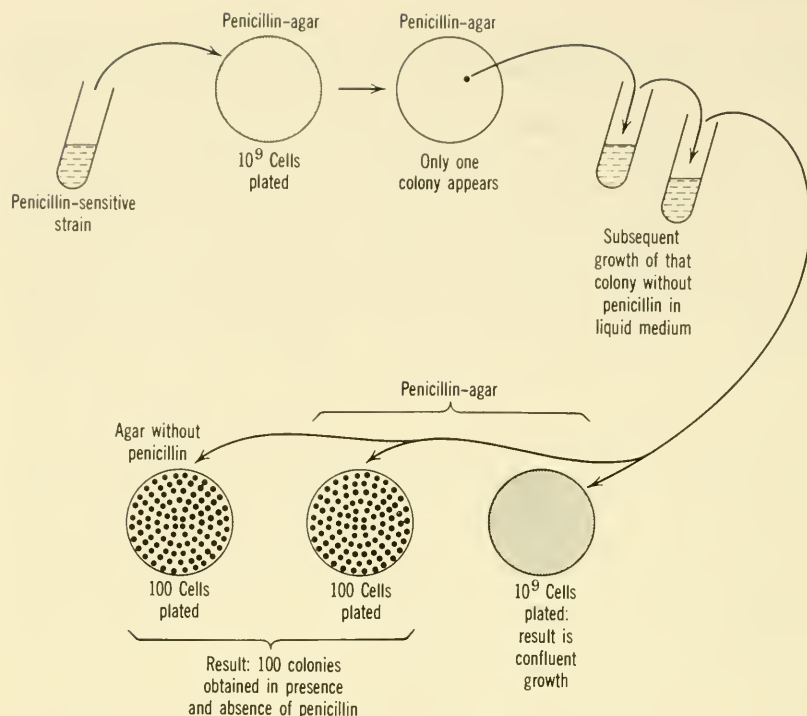


FIGURE 1.6. Selection of a drug-resistant strain. To select a drug-resistant strain, a heavy suspension of drug-sensitive cells is placed on agar containing the drug. Most cells die, and any colonies that appear are probably drug-resistant. They are further tested by growth for several generations in liquid culture in the absence of the drug, followed by plating again on drug-containing agar. If the strain is now resistant, a colony should form from every cell, both in the presence and in the absence of the drug. If a heavy suspension (e.g., 10^9 cells) of drug-resistant cells is plated on drug-containing agar, the growth will be confluent. The suspension must be diluted so that only about 100 cells are plated; the resulting growth will be in the form of discrete countable colonies.

individual drug-resistant cells arising by transformation can be isolated from a population of many million sensitive cells simply by plating the mixture on drug-containing solid medium, low frequencies of transformation can be measured with precision.

The results of a double transformation experiment are given in Table 1.3. When sensitive cells were treated with a DNA preparation from a penicillin-resistant, streptomycin-resistant strain, three classes of transformants were found: double transformants and two classes of singles.

The frequency of doubles was found to be approximately the product of the frequencies of the two classes of singles, indicating that the two DNA's are incorporated independently. They may therefore be considered separate genetic units and, in a formal sense, separate particles. Further evidence of the particulate nature of hereditary material comes from studies of the reversibility of transformation.

Reversibility of transformation

Independence of different genetic units in transformation is found frequently but not in all instances. Examples have been observed of linked transformation, that is, the recovery of double transformants more frequently than the product of the frequencies of the singles. This example is important both because it demonstrates *linkage* and also because it provides a method of demonstrating an important principle, namely, the reversibility of transformation.

Some pneumococci cannot utilize the sugar, mannitol, for growth, but mannitol-fermenting strains can be isolated. A streptomycin-resistant (*str-r*), mannitol-fermenting (*mtl*⁺) strain was prepared by serial transformation, and its DNA was applied to a streptomycin-sensitive (*str-s*), mannitol-nonfermenting (*mtl*⁻) strain. The results, listed in Table 1.4, show that the frequency of double transformants was much higher than the product of single transformations for each factor. This linkage makes it possible to investigate the question whether DNA from *str-s* cells will transform resistant cells back to sensitivity, and whether DNA from *mtl*⁻ cells will transform *mtl*⁺ cells back to the *mtl*⁻ condition.

The low frequency of transformation makes mandatory the use of some selective device to recover the transformed cells from the great excess of nontransformed ones. In transformation to drug resistance or to utilization of a particular compound for growth, the selective device is simply the use of media containing the drug or the particular growth compound. In the reverse transformation, the experimental technique employs

TABLE 1.4
Double Transformation with Linked Markers
(After Hotchkiss, 1954, Symposium on Genetic Recombination,
J. Cell Comp. Physiol., **45**:1)

Donor DNA	Receptor Cells	Transformants	
		Single	Double
<i>mtl</i> ⁺ <i>str-r</i>	<i>mtl</i> ⁻ <i>str-s</i>	<i>mtl</i> ⁺ <i>str-s</i>	0.01
			<i>mtl</i> ⁺ <i>str-r</i> 0.001-0.002
		<i>mtl</i> ⁻ <i>str-r</i>	0.01

TABLE 1.5
Reversibility of Transformation Using Linked Markers
 (After Hotchkiss, 1954, Symposium on Genetic Recombination,
J. Cell. Comp. Physiol., **45**:1)

Donor DNA	Receptor Cells	Selected Transformants	Double Transformants (as % of Singles)
<i>mtl⁺ str-s</i>	<i>mtl⁻ str-r</i>	145 <i>mtl⁺</i>	14.5% <i>mtl⁺ str-s</i>
<i>mtl⁻ str-r</i>	<i>mtl⁺ str-s</i>	134 <i>str-r</i>	12.0% <i>mtl⁻ str-r</i>

double transformants, as shown in Table 1.5. The experiment is set up so that single transformants of one class are selected on the appropriate medium, and then they are individually tested for the occurrence of the second transformation. Since there is 10–20 per cent linkage in this system, it is only necessary to test about five to ten times as many colonies as have been doubly transformed. The results of such an experiment indicate that there is about equal probability of transformation in either direction. This is true for both the streptomycin and the mannitol factors, and in analogous experiments with other determinants, similar results have been obtained.

In the transformation system, the method used to demonstrate unequivocally that a transformed cell has really been changed genetically is to test the transforming ability of its DNA. When a cell is transformed from streptomycin sensitivity to resistance, one may inquire whether the transformed cells (and their descendants) now contain only the new determinant, or whether they contain both the old and the new. The reversibility experiments demonstrate that transformation involves the replacement of the old determinant by the new. Furthermore, the yield of double transformants, resulting from linkage, is the same regardless of whether the change is from *mtl⁺ str-s* to *mtl⁻ str-r* or from *mtl⁻ str-r* to *mtl⁺ str-s*. This result indicates that the spatial relations responsible for linkage are unaltered by the condition of the marker, that is, whether it is *mtl⁺* or *mtl⁻*, *str-r* or *str-s*.

These experimental results lead one to view a hereditary determinant or gene as part of a DNA molecule capable of assuming at least two alternative states, only one of which can be present at a time. Recent studies of capsular type transformation have provided evidence that there are several alternative states of capsular determinants which can replace one another but do not coexist. These alternative states are called *alleles*.

Relation between the gene and the DNA molecule

A further clarification of the physical meaning of linkage observed in transformation experiments, and of the relation between unit determinants or genes and DNA molecules, has come from recent studies of the physical chemistry of DNA. It was discovered that the DNA double helix collapses upon heating, as a result of breaking of hydrogen bonds between the nitrogen bases, and that the collapsed molecules lose their transforming activity. As may be recalled from Figure 1.3, there are two hydrogen bonds between adenine and thymine, and three between cytosine and guanine. Probably for this reason, the temperature at which DNA molecules collapse is directly related to their guanine-cytosine content; the higher the G-C content, the higher is the so-called melting out temperature.

By carefully controlling the temperature to which a purified preparation of pneumococcal DNA is heated, it has been found possible to inactivate one genetic marker before another, as shown in Figure 1.7. For example, there is a great loss of transforming activity by the gene for resistance to the drug, micrococcin, at a temperature which hardly affects the gene for streptomycin resistance at all. However, the activity of two genes involved in sulfanilamide resistance and that of the gene for

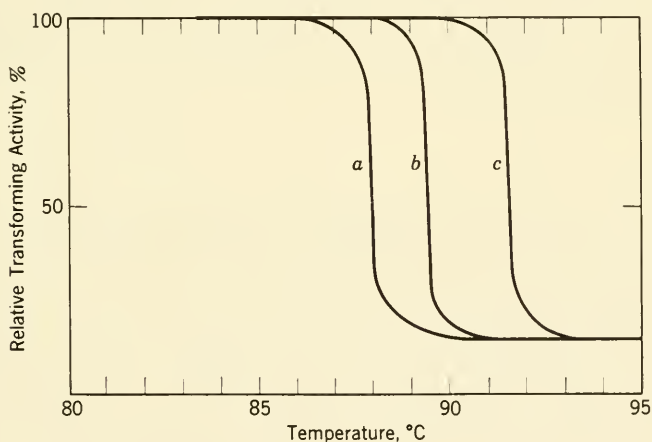


FIGURE 1.7. Inactivation temperatures of different transforming factors. Under standardized conditions, transforming ability of purified DNA extracts is lost after heating to temperatures which are characteristic for particular genes in pneumococcus.

- (a). Micrococcin resistance is lost at 88° C.
- (b). Resistance to streptomycin and two closely linked genes which determine sulfanilamide resistance (*Fa* and *Fad*) are lost together at 89.5° C.
- (c). Amethopterin resistance is lost at 91.5° C. (from M. Roger, unpublished).

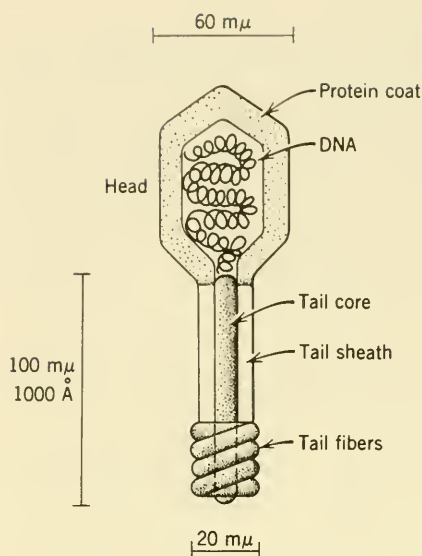


FIGURE 1.8. Structure of *T2* bacteriophage (from Kozloff, in: Zirkle, A Symposium on Molecular Biology, Chicago, Copyright (1959) by the University of Chicago, p. 178).

streptomycin resistance are all lost together; and in transformation studies these three genes exhibit linkage. Thus the parallel loss of activity by linked genes and independent loss of activity by unlinked genes, at characteristic melting out temperatures, are very fine evidence that linked genes are on the same molecule and unlinked genes are on different molecules of DNA.

Phage DNA

Another system in which the DNA composition of hereditary material has been demonstrated is that of certain bacterial viruses, the *T* phages, which attack the bacterium, *Escherichia coli*. These viruses, or phages, have a complex structure composed primarily of protein and DNA (Fig. 1.8). All of the DNA is in the head of the phage, and the tip of the tail contains a specific protein which attaches to particular receptor sites of the bacterial wall. After infection, multiplication occurs in the bacterial host cell, and a large number of progeny phages, genetically identical with the infecting one, are produced. Phages may differ from one another by a number of heritable properties which are transmitted to their progeny. Thus the phage carries hereditary determinants.

One may then ask: Can the hereditary determinants be identified with a particular chemical constituent of the phage? This question was answered by the following experiment.

Phage DNA can be made radioactive by infecting bacteria grown on media containing $[P^{32}O_4]^-$. The radioactive phosphate assimilated into the bacteria is passed on to the newly forming progeny phages. Phage proteins do not contain phosphorous, so only the DNA is labeled. Similarly, phage protein can be selectively labeled with radioactive sulphur, S^{35} , by growing the host bacteria on $[S^{35}O_4]^-$, since DNA does not contain sulphur. This method of differential labeling makes it possible to distinguish between phage DNA and phage protein after infection. Normally after infection one finds both labels associated with the bacteria. However, Hershey and Chase, at the Carnegie Laboratory of Genetics at Cold Spring Harbor, found that, if the bacteria were agitated in a Waring Blendor immediately after infection, most of the P^{32} label was associated with the cells, and the S^{35} was largely in the surrounding medium. The interpretation of these results is diagrammed in Figure 1.9. They postulated that, immediately after attachment of the phage to

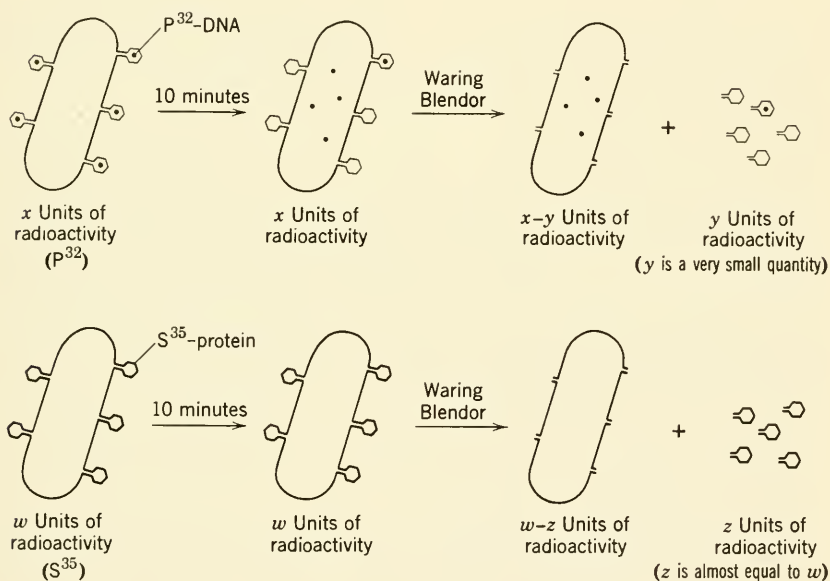


FIGURE 1.9. Demonstration that only phage DNA enters the bacterial host cell after infection. Cells are infected with P^{32} -labeled phage, and after being allowed time for infection, they are agitated in a Waring Blendor which shears off the phage coats. Radioactivity is measured at each step in the procedure. Very little of the original P^{32} radioactivity is lost from the cells. Thus, the phage DNA has been incorporated into the host cells. When phage are labeled with S^{35} in their proteins, and the same experiment performed, the results are very different. Most of the radioactivity is found in the supernatant with the phage protein; very little enters the cells.

a site on the bacterial wall, the DNA enters the cell, but the coat remains outside, attached to the wall. In the Waring Blendor, the coats are torn off the cells by shearing forces and remain in the suspending liquid. The bacteria are not harmed by the blendor treatment, and phage multiplication is normal in these cells. This interpretation has been fully substantiated by electron micrographs of the infection process.

This experiment demonstrates beautifully that it is primarily the DNA which enters the host cell, leaving most of the protein outside. Nonetheless, a nonsulphur containing protein, amounting to some 1–2 per cent of the total, has been found in association with the entering DNA. This protein is a very simple one, consisting mainly of the amino acids, lysine, glutamic, and aspartic acids, and more likely represents a nonspecific carrier of the DNA than hereditary material.

Although this experiment itself does not provide a decisive demonstration of the postulated role of DNA as hereditary material, there are many supporting data from other experiments with the phage system fully consistent with this hypothesis. For example, strong support comes from the analysis of lethal effects of incorporated P^{32} resulting from atomic disintegrations within frozen, stored phage.

Tobacco mosaic virus

Another system which provides evidence of the nucleic acid composition of hereditary material is that of the tobacco mosaic virus (TMV). TMV contains about 6 per cent ribonucleic acid (RNA), the rest being protein. It has been shown in a number of experiments performed independently by Schramm in Germany and by Fraenkel-Conrat in California that the TMV hereditary determinants are carried in the RNA. A method was developed for separation of RNA from protein, and it was demonstrated that the RNA alone is infectious; cells infected with this naked RNA produce new virus particles, complete with their protein envelopes.

Although the RNA infection experiment demonstrates that hereditary information can be carried by RNA alone, one may still ask whether the protein moiety may also carry information. The following experiment was designed to answer this question.

In chemical studies it was observed that the RNA itself is highly unstable, but that it could be stabilized by adding a protein, not necessarily TMV protein. One protein which worked well was that extracted from a related virus, Holmes rib-grass virus (HRV). Similarly, the RNA of HRV is infectious, but more successfully so if stabilized with a protein, such as that from TMV. Thus it is possible to make two

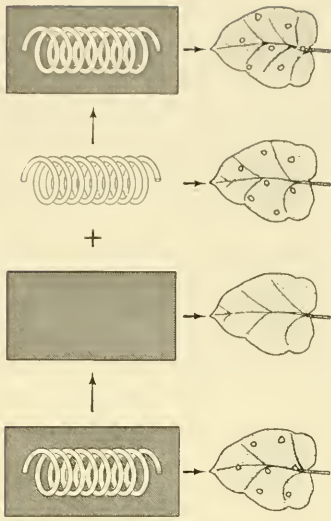
synthetic strains of virus, each with the RNA of one strain and the protein of the other. When a plant is infected with one of these synthetic strains, new progeny virus particles are produced. These particles can then be classified by the type of infection they produce as either TMV, HRV, or something atypical. The results of such an experiment, diagrammed in Figure 1.10, show clearly that the RNA alone determines the type of virus progeny produced. A similar conclusion has been drawn from infectivity studies with isolated RNA from a number of animal viruses, notably those producing polio and encephalitis.

One may conclude from these experiments that two classes of macromolecules, the ribonucleic acids and the deoxyribonucleic acids, carry hereditary information in particular systems, the transformable bacteria and the viruses. What reasons are there to consider these findings of general significance and applicable to higher forms?

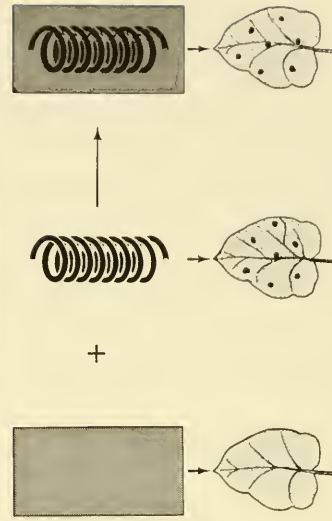
Nucleic acids in higher forms

There is a considerable body of information available concerning the localization of nucleic acids in specific regions within cells. An electron micrograph of a cell is shown in Plate I. DNA is located exclusively within the nucleus and is entirely a chromosomal constituent (except in special circumstances to be discussed later). The importance of chromosomes as carriers of hereditary material was proposed in the last century on the view that the meticulous mechanics of their segregation at cell division must reflect the tremendous importance of each chromosomal element in the life of the cell. Subsequently, with the development of genetics and the correlations between genetic and cytological observations, these inferences of the early cytologists were abundantly confirmed (see Chapter 4). More recently, with the cytochemical localization of DNA in the chromosomes, it was proposed that DNA itself might represent an essential constituent of hereditary material. Without constituting any proof of the genetic role of DNA, this cytochemical evidence could certainly be taken as consistent with it.

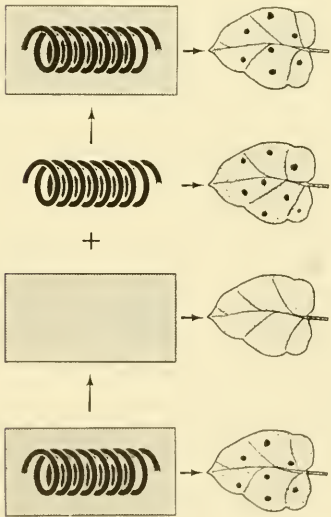
Further indirect evidence came with the demonstration that the total DNA per cell is constant for each species. This is true irrespective of the tissue examined except that sex cells, the egg and sperm, which have half the number of chromosomes of other cells also have half the DNA content (Table 7.1), and special cells with double or triple the usual chromosome count have a corresponding increase in DNA content. The constancy of DNA is impressive because of its generality. Nonetheless, it should be pointed out that a particular class of chromo-



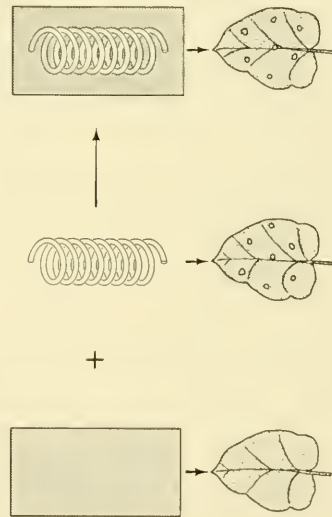
(b)



(d)



(a)



(c)

somal proteins—the histones—is maintained with the same constancy as DNA, but there is no evidence from other kinds of data that the histones play a role as hereditary determinants.

RNA is found in many places within the cell. In the nucleus, both the chromosomes and the nucleolus contain RNA. At least two distinct RNA fractions have been isolated from the cytoplasm, and one of them, located in the microsomal particles (ribosomes), has been shown to vary greatly in amount from tissue to tissue. There are probably many other RNA fractions as yet unidentified. Indeed, most information available about the distribution and function of RNA fractions is very recent and fragmentary.

With respect to a genetic role for RNA, as yet the only critical evidence comes from virus infection experiments of the type just described. There is genetic evidence for the existence of hereditary determinants which are not chromosomal and possibly not DNA in composition. One may postulate RNA as the carrier of this type of hereditary information, but there is no direct confirmation of this hypothesis as yet. Genetic and chemical evidence about nonchromosomal determinants will be taken up in detail in Chapter 9.

Some indirect evidence of a genetic role of nucleic acids in all organisms came with the demonstration that cells can be killed or genetically altered by irradiation with ultraviolet light, and that the most effective wave lengths are those at which the specific absorption of nucleic acids is maximal. The results of some typical experiments, shown in Figure 8.13, do not distinguish between DNA, RNA, free nitrogen bases, nucleosides, and nucleotides, since their UV spectra are all quite similar. There is the further problem, always a difficulty in the interpretation of such action spectra, that the compounds which absorb the energy may simply pass it on to other compounds which are the biologically relevant ones. Thus, these experiments do not critically demonstrate the genetic role of nucleic acids, but they are consistent with such a view.

FIGURE 1.10. Demonstration that RNA alone is the genetic material of the tobacco mosaic virus. The RNA core can be dissociated from the protein coat and recombined with the same or a related protein. Both the dissociated and reassociated forms are infectious. Protein coats alone are not infectious. (a) and (b) show the different lesions produced on the tobacco leaf by related forms of virus. In (c) and (d), the RNA of one virus has been reassociated with the protein of the other. In both combinations, the RNA component determines the type of lesion, and thus the genetic continuity of the virus.

The question of protein as hereditary material

Until recently, it was the prevailing view that of all the cellular constituents, only proteins were complex enough to qualify as hereditary material. The work with bacteria and phages has provided convincing evidence that nucleic acids can play this role. But the question still remains whether proteins also are represented among the hereditary determinants of the cell. There is virtually no evidence in direct support of such a possibility, but neither is there anything decisively against it. The best negative evidence comes from the experiments with synthetic plant viruses, in which the nature of the protein coat had no apparent genetic effect upon the progeny viruses. Protein transformations in bacteria do not work, but in this field negative results have little weight.

GENES AND GENE ACTION

In this chapter we have discussed experiments in which the chemical nature of hereditary material was demonstrated. Two levels of analysis were necessary in these experiments, one dealing with the chemistry of the material, and the other with its biological role. Transformation experiments have given us a general correlation of nucleic acid molecules with genetic determinants, but have told us nothing of the detailed chemical composition of individual genes, nor of the manner in which they exert their manifold effects upon cell development.

To specify the detailed chemistry of a gene will require advances both in chemistry and in genetics. The chemical problem, involving isolation of individual nucleic acid molecules and analysis of their nucleotide sequences, appears formidably difficult. On the other hand, the genetic problem of identifying the biological unit is advancing rapidly at the present time, and is even providing material to aid in unraveling the chemistry of the gene. Genetics began with the demonstration by Mendel that hereditary material could be analyzed into sets of genetic units by means of breeding experiments. Recent advances in genetics have provided refinements in the concept of the genetic unit and new tools for its study.

What is a gene? We define the gene in the broadest sense: a hereditary determinant which in its alternative forms is responsible for differences in a particular trait. We do not specify either its location in the cell (chromosomal, nonchromosomal) or its constitution (DNA, RNA, protein) because flexibility and generality are necessary in the face of

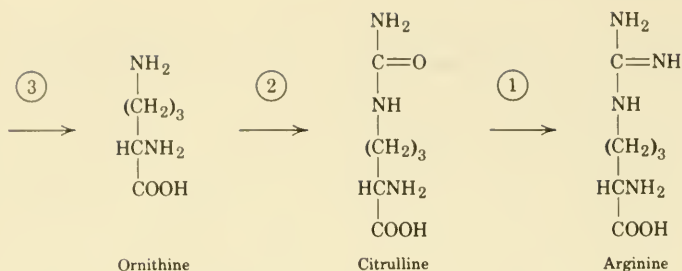
the rapid advances being made in this field. In actuality, most genetic investigations have been undertaken with chromosomal genes, particularly in the studies of recombination. In studies of mutation, the genetic elements have not always been identified.

The existence of the gene has been inferred from observations of differences in heritable traits of individuals in a pedigree. Thus observations of altered phenotypes have provided the raw material from which the science of genetics was constructed. Before proceeding to a more detailed analysis of the genetic unit, then, let us summarize what is known of the action of genes in determining the phenotypes by which they are identified.

How do genes act? When one observes the extraordinary precision of heredity—the extent to which progeny resemble their parents, embryos develop identically, species differ consistently—one feels convinced that the directing role of hereditary determinants must be pervasive, encompassing a great variety of activities. The perpetuation of the complexities of subcellular organization, the synthesis of small molecules and of specific macromolecules at the right time and in the right place, must demand a very high level of integration; presumably all this is under gene control. Viewed from the vantage point of the finished product, the analysis of gene action looks bewilderingly complex.

On the other hand, if one considers the problem of gene action in a single-celled microorganism, and further simplifies matters by surveying the effects of known gene mutations, the picture becomes somewhat less blurred. This approach was developed in a forceful way with the systematic investigation of mutants of the bread mold, *Neurospora*, by Beadle and Tatum. They, and subsequently many others, isolated mutant strains called *auxotrophs*, each of which had acquired, as a result of a single gene mutation, a growth requirement for some particular compound, generally an amino acid, vitamin, purine, or pyrimidine, not required by the parental strain. These mutants were studied biochemically to locate the metabolic effect of each mutation.

For example, in one experiment, fifteen mutant strains were isolated which required arginine for growth. Biochemists supplied the information that the last few steps in arginine synthesis in mammalian liver involved the compounds, ornithine and citrulline, as shown in Figure 1.11. The mutants, tested for their ability to grow with any of these compounds, fell into three groups: those which could use only arginine (Group 1), those that could use either arginine or citrulline (Group 2), and those that could use any one of the three compounds equally well for growth (Group 3). It was further noted that mutants of Group 1 accumulated citrulline, supporting the growth of Group-2 mutants which,



Mutant	Growth on			Position of block
	<i>orn</i>	<i>citr</i>	<i>arg</i>	
<i>a</i>	+	+	+	3
<i>b</i>	-	+	+	2
<i>c</i>	-	-	+	1

FIGURE 1.11. Terminal steps in arginine biosynthesis. The position of genetic blocks in this pathway in various mutant strains can be found by comparing growth on various intermediate compounds. In general, mutant strains grow on intermediate compounds located beyond the blocked step.

in turn, accumulated ornithine which could be utilized by mutants of Group 3.

In this experiment it was possible to localize the phenotypic difference between wild-type and each of the fifteen mutants at a particular biochemical reaction; this was achieved both by the accumulation test and by arranging the mutants in a logical sequence with respect to growth requirement. The fact that the inferred sequence coincided with that established by biochemical methods provided a neat confirmation of the biochemical findings, and assurance that the mutations had blocked steps in a normal pathway. In the twenty years since this method was developed, it has provided a wealth of information of value both to genetics and to biochemistry. Auxotrophic mutants have provided biochemists with a fast and easy method to investigate unknown biosynthetic pathways by testing the effects of suspected intermediates upon the growth of mutant strains; a flood of new data has been forthcoming.

The impact of auxotrophic mutants upon the field of genetics has been profound. Mutations could be selected to block most of the known enzyme-catalyzed steps in the biosynthesis of cell constituents. Beadle and Tatum generalized these findings into a hypothesis of gene action which states that (1) gene function consists of "imposing specific configurations on protein and other molecules in the final step in their

synthesis," and that by so doing, "genes directly determine enzyme specificities and thereby control in a primary way enzymatic syntheses and other chemical reactions in the organism;" and (2) "that a given enzyme will usually have its final specificity set by one and only one gene."

This hypothesis involves a number of important assumptions. In the first place, there is a conceptual jump from the mutant phenotype to the function of the unmutated allele, in the assumption that the activity of the unmutated form of the gene is to carry out the reaction which is blocked in the mutant. Secondly, it is assumed that the gene-determined effect is upon the enzyme which catalyzes the reaction blocked in the mutant; and thirdly, that the gene influences the enzyme solely by determining its specificity. Finally, it is proposed that a one-to-one correlation exists between each gene and a particular enzyme.

This hypothesis can be considered more concretely with the aid of an example based upon studies by Horowitz and co-workers. In *Neurospora*, some strains produce the black pigment, melanin, only when grown at temperatures of 25° C or lower, while other strains produce the pigment at all temperatures. This difference is correlated with a single gene difference; the allele T^L is carried in temperature-sensitive strains, and T^S in the others. T^L strains contain a thermolabile form of the enzyme *tyrosinase*, which catalyzes one step in melanin formation, and T^S strains contain a thermostable form of the enzyme. Thus, the phenotypic difference between strains results from alterations in a physical property of an enzyme. Two other genes, *ty-1* and *ty-2*, are known which also affect tyrosinase activity. When the mutant form of either *ty-1* or *ty-2* is present, no tyrosinase is formed, unless the strain is grown on a special medium, in which case large amounts of the enzyme are produced. Neither *ty-1* nor *ty-2* appears to alter the properties of tyrosinase; when formed, its thermostability is determined by whichever T allele is present.

This example supports the one-gene-one-enzyme hypothesis insofar as the blocked reaction can be attributed to absence or alteration of the enzyme, tyrosinase. However, not one but three genes have been found which influence the appearance of this enzyme, and apparently they act in different ways. T influences enzyme structure, whereas *ty-1* and *ty-2* appear to exert their influence upon enzyme formation. Other mutant systems provide evidence consistent with this picture: that the controls of enzyme formation, of enzyme activation, and of specificity are all devices in the *armamentarium* of gene action. However, the precise mechanisms by which the genes bring about these effects are hardly known at all.

In the absence of more direct information, certain formal considera-

tions are of interest. Gene action can be viewed as the transfer of genetic information from nucleic acids to other cell constituents. This is a *coding* problem; the nucleic acids may be considered a tape coded with signals which determine cellular activities. The only elements of the nucleic acid molecule that appear suitable for coding are the nucleotides themselves. Since there are four different nucleotides regularly present, we can consider the nucleic acid alphabet as composed of four letters. [One may also consider a binary code with only two letters, determined by whether there is a keto or an amino group on the 6-position in the nitrogen bases (see Figure 1.1).]

Which cell constituents decode the tape? If we restrict our considerations to genes which act by controlling protein specificity, then we can restrict the coding problem to translation from nucleic acids (two- or four-letter alphabet) to proteins, which have a twenty-letter alphabet (most proteins contain twenty different amino acids). According to present biochemical information, most proteins are synthesized on ribonucleoprotein particles in the cytoplasm, in which, so far as is known, no DNA is present. Consequently, it has been postulated widely that the last steps in protein synthesis involve translation from RNA to protein, and a number of codes have been proposed to solve this translation step. Since DNA and RNA are so similar and the codes devised so general, they can be considered purely as nucleic-acid-to-protein codes without specifying which nucleic acid is involved. Despite the ingenuity of these codes, there is insufficient information available as yet for choosing among them.

Although there are many gaps in our knowledge, we may be sure from what we have already learned that genes must be fairly large. If their influence is upon protein structure or the rate of protein synthesis, each must contain enough information to relate it to a particular enzyme or enzyme-forming site, and not to any others. Analogous considerations would apply to other gene functions. In our considerations of the gene we shall assume, therefore, that the functional unit consists of many nucleotides, numbering perhaps in the hundreds. Later (Chapter 6) we shall see that this conception has an independent experimental basis.

BIBLIOGRAPHY

- Avery, O. T., C. M. MacLeod, and M. McCarty, 1944. Studies on the chemical nature of the substance inducing transformation of pneumococcal types. Induction of transformation by a desoxyribonucleic acid fraction isolated from pneumococcus Type III. *J. Exptl. Med.*, **79**:137-158.
- Beadle, G. W., 1945. Biochemical genetics. *Chem. Rev.*, **37**:15-96.

- Beadle, G. W., 1945. Genetics and metabolism in *Neurospora*. *Physiol. Rev.*, **25**:643-663.
- Chargaff, E. and J. N. Davidson, 1955. *The Nucleic Acids*, Vol. II. New York, Academic Press (the articles by Swift, Vendreley, Hotchkiss and Brachet).
- Davidson, J. N., 1957. *The Biochemistry of the Nucleic Acids*. London, Methuen and Co., Ltd., 248 pp.
- Fraenkel-Conrat, H., B. A. Singer, and R. C. Williams, 1957. The nature of the progeny of virus reconstituted from protein and nucleic acid of different strains of tobacco mosaic virus. McElroy, W. D. and B. Glass, (ed.). *Chemical Basis of Heredity*. Baltimore, Johns Hopkins Press, pp. 501-517.
- Fraenkel-Conrat, H., 1959. Structure and infectivity of tobacco mosaic virus. *Harvey Lectures*, **53**:56.
- Hershey, A., and M. Chase, 1952. Independent functions of viral protein and nucleic acid in growth of bacteriophage. *J. Gen. Physiol.*, **36**:39-56.
- Horowitz, N. H., M. Fling, H. L. Macleod, and N. Sueoka, 1960. Genetic determination and enzymatic induction of tyrosinase in *Neurospora*. *J. Mol. Biol.*, **2**:96-104.
- Hotchkiss, R. D., 1955. The genetic chemistry of the pneumococcal transformations. *Harvey Lectures*, **49**:124-144.
- Hotchkiss, R. D., 1955. Bacterial transformation. In; Symposium on Genetic Recombination. *J. Cell. Comp. Physiol.*, **45**, Suppl. 2:1-22.
- Oncley, J. N., 1959. *Biophysical Science*. New York, Wiley (the articles by Levinthal and Rich).
- Wagner, R. P. and H. K. Mitchell, 1955. *Genetics and Metabolism*. New York, Wiley, Chapters 4 and 5.
- Watson, J. D. and F. H. C. Crick, 1953. Genetic implications of the structure of deoxyribose nucleic acid. *Nature*, **171**:964.
- Wilkins, M. H. F., 1956. Physical studies of the molecular structure of deoxyribose nucleic acid and nucleoprotein. *Cold Spring Harbor Symp. Quant. Biol.*, **21**:75-90.

2 The mutable unit of heredity

If all organisms were alike, the question of inheritance might never have arisen. That "like produces like" would be the natural order of things. But were the question to arise in the ranging mind of some philosopher, the science he would found would be a most difficult one. It would involve the question: What is it about organisms that makes them develop identically? This is, in fact, the problem of embryology, and its answer is as important as it is elusive. But organisms do differ, so that we can ask the question in another way: What is it about one organism that makes it different from another? It is the differences that provide the observational units of genetics. It was the analysis of the inheritance of differences among garden peas that led Mendel to the discovery of his laws. Similarly, when an unusual white-eyed fruit fly was captured in a Columbia University laboratory, it was bred with its red-eyed relatives as the first of a classic series of experiments by Thomas Hunt Morgan and his students that led to the establishment of the chromosomal theory of heredity.

Differences among organisms of the same species are not only indispensable for the study of heredity but are also the stuff from which new species are made. The mutations that initiate these differences are the driving force in evolution. They provide the raw material for the interaction of the processes of heredity with selective forces in the environment; thus they permit the change that is so characteristic of life. It has been supposed that mutations yield only harmful, inferior characters. As a matter of fact, studies on organisms "higher" than bacteria show that this is often the case. But we must remember that organisms as we find them have already been highly perfected during evolution. Mutant genes of most kinds must have been tested as an assemblage for survival

value. The present genotype is likely to be nearly the best for existing circumstances. Consider a Swiss watch: to hit it with a hammer is more likely to impair its function than to improve it.

Nonetheless, we may suppose that evolution continues with the selection of improvements in the hereditary material, by the reorganization of the whole genotype and through changes that occur in the environment. That advantageous mutations do occur is a well-established fact. In the fruit fly, for example, some mutants are inferior in the environment in which they arose but superior in other environments. The way in which new species arise is another story which will not be treated in this book. It is part of the science of evolution, which, through the use of genetic techniques, has become one of the most active and interesting branches of biology.

In the next chapter we shall take up the way in which differences are transmitted from parent to offspring; this chapter is concerned with the origin of observed variation. Novel characters of a heritable kind arise as a consequence of sudden changes in the genetic material. Such *mutations* are rare. It is thought that one took place in Queen Victoria of England. Although there is no record of hemophilia among her ancestors, she transmitted a factor for that disease to her offspring. Not much has yet been done to study the mutation process in man. Experimentation is somewhat easier in organisms such as the fruit fly, *Drosophila*, which has a generation time of about two weeks and can be inbred by brother-sister mating for many generations to yield individuals with almost identical genetic constitutions. In such pure lines, a new type having, say, white eyes instead of red may suddenly appear with a frequency of about one in a hundred thousand.

Mutation may be studied much more conveniently among organisms which give rise by asexual reproduction to billions of offspring in one day, as do bacteria and viruses. Such microorganisms are also convenient for chemical studies and, as we shall see, have genetic systems *basically* similar to those in all other organisms. As a matter of fact, the possession of genetic material which can mutate is a prerequisite for life.

THE PARTICULATE NATURE OF THE MUTABLE UNIT

Mutation is a heritable change in the genetic material; a *mutant* is the changed organism that results. The standard of reference is the wild-type condition, the state of organisms as they are found in nature. Obviously this definition is arbitrary because even in nature organisms and their hereditary materials vary. But once some condition has been

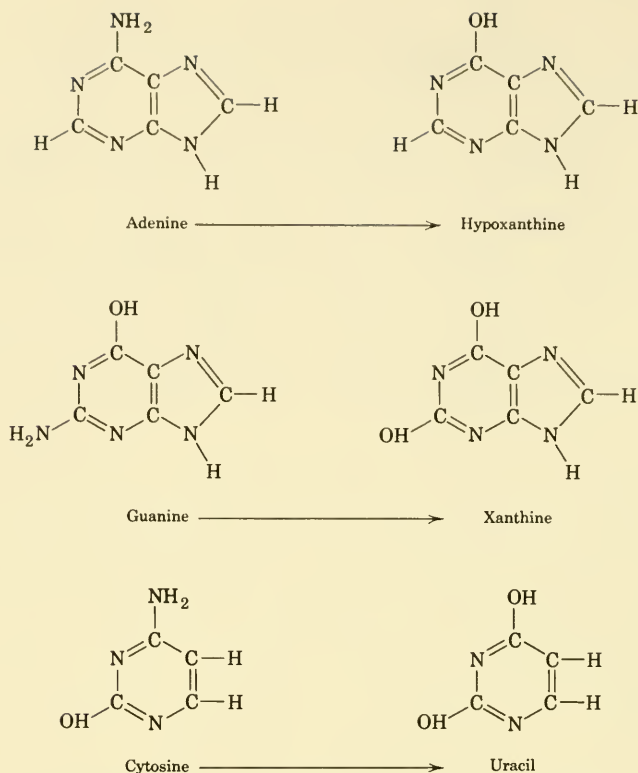
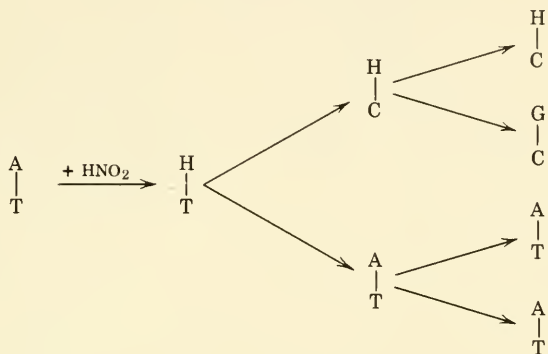


FIGURE 2.1. The changes in bases resulting from oxidative deamination caused by treatment with nitrous acid.

designated wild type, any heritable variation from it is called mutant. In some mutation studies, the mutated elements have been localized in particular regions of chromosomes and even within the DNA. There are also instances of nonchromosomal inheritance and, although the chemical basis of this kind of heredity has not been determined, it is probably not DNA. The ability of RNA to carry hereditary information as established by work with plant and animal viruses may represent a general feature of cell heredity.

The fact that RNA and DNA are both polynucleotides provides a common denominator accounting for some similarities in their mutability. For example, nitrous acid acts as a mutagen for both substances because, by oxidative deamination, it substitutes an hydroxy for an amino group and converts adenine into hypoxanthine, guanine into xanthine, and cytosine into uracil (Figure 2.1).

Hypoxanthine and xanthine are expected to have, in replicating DNA, pairing properties like those of guanine; uracil should be like thymine. As a result, following deamination and two subsequent replications, a mutation might occur in the following way:



When the RNA of tobacco mosaic virus is isolated from its protein, treated with nitrous acid, and allowed to infect a tobacco leaf, mutant TMV are produced. When a treatment was used which inactivated about half of the virus population, the number of mutants increased some twentyfold over the number present in the untreated control. This absolute increase in the number of mutants cannot be attributed to a selective survival of pre-existing mutants in nitrous acid, for their number could not, in that case, have increased above that initially present. The new mutants must have been induced by the nitrous acid.

The same effect is found with DNA which may be extracted from drug-sensitive pneumococci, treated with nitrous acid, and then tested for its ability to transform sensitive cells to a resistant condition. The nitrous acid treatment caused mutations to a streptomycin-resistant condition in DNA from streptomycin-sensitive bacteria. Once again, an absolute increase in the number of mutant molecules was observed.

The dose of mutagen may be increased by exposing the RNA or DNA to the same concentration of nitrous acid for increasing periods of time. With this procedure, the rate of production of mutants may be constant with time (e.g., Figure 2.2). Such kinetics, in which each successive and equal unit of dose induces the same number of mutants, indicate that a one-event process is at work. Were two chemical events required for mutation, the first small exposure would mostly bring about only one reaction per cell; in very few cells would both events occur. Further treatment would induce the second event in cells that had already undergone the first reaction. As a consequence, the rate of production

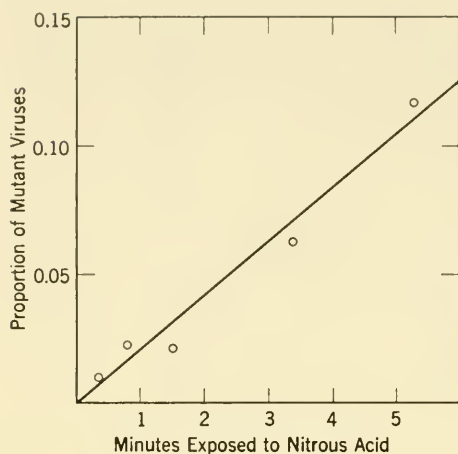


FIGURE 2.2. The rate of production of necrotic mutants detected by the infection of tobacco leaves with the RNA of tobacco mosaic virus which had been exposed to nitrous acid (after Mundry and Gierer, 1958, *Z. Vererbungslehre*, **89**:614).

of mutants would increase with time. But in the nitrous acid experiments the rate of production of mutants is constant, and so we conclude that one chemical reaction is sufficient to cause a mutation. This does not mean that every oxidative deamination of a base in RNA causes a mutation, but rather that when a suitable base is changed the mutation takes place. Nor does it mean that all mutations are caused by a single modification of a single base in the polynucleotide chain. As we will see later, mutations may occur in various ways, including even gross rearrangements of many bases in fairly large segments of the chromosome.

The fact that the change of one base can cause a mutation points up an interesting conceptual situation. The unit that is being studied in mutation is at least as small as a single nucleotide, but the gene must be considerably larger than that. In order to possess the specificity necessary to account for gene action, the gene as a functional unit of genetic material must consist of many nucleotides; it is only in a polynucleotide that different base sequences can be achieved. The smallest mutable unit is, therefore, smaller than the unit of function, and only a part of it. From this consideration alone it is apparent that genetic material consists of at least two kinds of units, namely, functional units (genes) and mutable subunits of which the genes are composed.

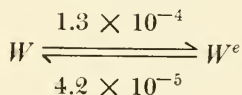
Subdivision of the genetic material may be studied by still other operations. For example, we saw that certain genes are often linked in transformation but that, nonetheless, they will sometimes be incorporated separately. We may ask: What is the smallest unit that can be separated from the neighbors to which it is linked? This question must

be studied by methods that examine genetic exchange; the unit of mutation must be investigated by methods that examine stability; and the unit of function must be studied by methods that examine the influence of the gene on the phenotype. Our knowledge of each unit is based upon a different experimental operation. In Chapter 6 we shall explore further the relationship between these concepts, but, in the meanwhile, when we consider any experimental operation, we must be sure to discriminate among the concepts we may employ. In this chapter, for example, the experiments reported relate to the mutable unit whose properties we will now examine.

THE REVERSIBILITY OF MUTATION

Muller and Stadler first showed, by the use of radiation, that mutations could be induced. Later Auerbach showed that chemicals can also be mutagenic. These discoveries led to the hope that the mutation process could be directed but, although a large number of mutagens are now known, they all increase the rate of mutation of many different mutable units in a nonspecific way. There is, then, a kind of randomness about the mutation process in that it is impossible to predict which mutant will next arise. Take, for example, nitrous acid which is specific at a chemical level; it oxidatively deaminates only those bases which have an amino group. But it is essentially nonspecific at a biological level, probably because genes contain so many nucleotides that a particular base change is as likely to occur in one gene as in another.

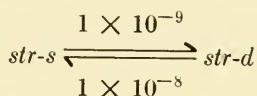
Mutation does not occur unidirectionally, carrying the phenotype from one state to another in series. Rather the phenotype oscillates back and forth among the different alternative states it can take. Studies by the Russian scientist Timofeeff-Ressovsky, working in Germany, showed that eye color in *Drosophila* could mutate from the normal bright red condition (W) to a paler eyed one (W^e) and back again.



The numbers along the arrows are the frequencies, (e.g., a frequency of one in a million, $1/10^6 = 10^{-6}$) of the designated mutants among the sperm. *Forward mutations* from the wild type to the mutant condition are here more frequent than *back mutations*.

It is with microorganisms that the reversibility of mutation can be studied most easily, for among them it is relatively easy to select either

forward or back mutants by manipulating the environment. Let us consider, for example, mutants of the intestinal bacillus, *Escherichia coli*, showing resistance to streptomycin. Even if they exist among streptomycin-sensitive cells (*str-s*) in a frequency as small as one in a billion (10^{-9}), they may be found simply by spreading several billion *str-s* cells on a plate of agar containing nutrients and streptomycin. Only the *str-r* mutants will grow to form visible colonies; the *str-s* cells will die. Among the resistant bacteria selected in such experiments, some—streptomycin-dependent (*str-d*)—actually require streptomycin for growth. When these *str-d* bacteria are spread on mutant agar without streptomycin, only the rare *str-s* bacteria among them grow and form colonies. These back mutants do not need streptomycin for growth and are no longer resistant to it. They may be selected among billions of *str-d* cells. In this way the mutation process is shown to be reversible.

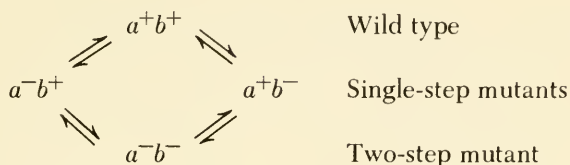


The numbers along the arrows here indicate the *rate* of mutation. We may consider the *str-s* state as the wild type even though in this case it is the more stable.

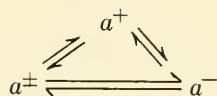
AN OPERATIONAL DEFINITION OF THE MUTABLE UNIT

In the transformation experiments we saw that the genetic material existed in mutually exclusive states, either *str-s* or *str-r*, and either *mtl*⁺ or *mtl*⁻. These alternative states of individual genes are called *alleles*. Members of the same allelic pair do not coexist in the same bit of genetic material but can only replace one another. But the same bit of genetic material contains individual members of different allelic pairs so that the replacement of one of these by its allele in transformation results in recombination. We have also seen that, when the genetic material is studied by the method of mutation, it is observed to pass back and forth between alternative states. It is not surprising that the fundamental genetic property of mutual exclusion of alleles should be observed by both methods since the alleles studied in transformation initially arose by mutation. Clearly then, the genetic material consists of mutable units which in their different states have different effects on the phenotype. By what experimental operations can we distinguish one mutable unit in its various allelic states from another mutable unit?

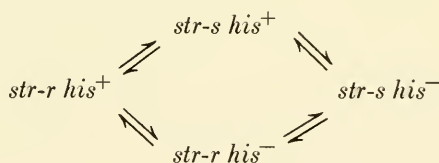
Consider for a moment what would be expected of two mutable units, each in two alternative states. We can see that there are four possible combinations:



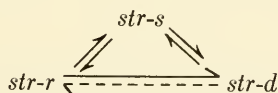
Because each of the units mutates independently, two mutational steps are required for the genetic material to pass from one condition to the opposite (e.g., from a^-b^- to a^+b^+). If only one mutable unit is involved and there are more than two states, each may mutate to another in one mutational step:



Now in practice, of course, we observe only phenotypes. If two unrelated phenotypes, such as growth on streptomycin and the ability to make histidine, are involved, there are four possible phenotypes. If among them we find such a relation as

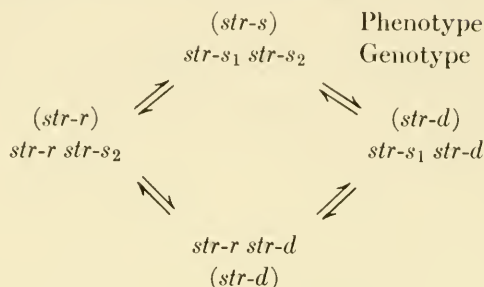


we can be sure that the pattern is caused by the mutation of two mutable units, not one. Sometimes what may seem to be three mutually exclusive phenotypes are found in the following pattern:

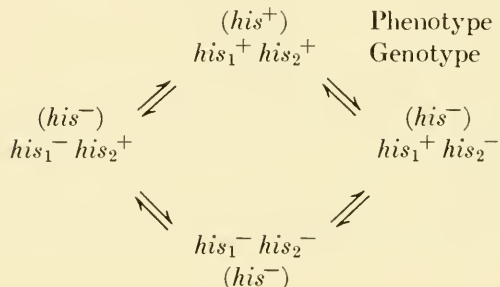


It could be that one mutable unit is here being observed in three of its states. On the other hand, it may be that there are two indistinguishable

str-d states so that the relation is really rectangular because two, not one, mutable units underlie the phenotypes:



Furthermore, when there are only two alternative phenotypes such as his^- and his^+ , we cannot so readily be sure that only one mutable unit is involved, for the relation may really be:

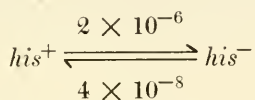


It is clear then that, as usual, it is more difficult to show that two things are identical, than that they are different. By a more careful examination of the phenotypes, it is sometimes possible to discover small differences between, say, the $his_1^- his_2^+$, $his_1^+ his_2^-$ and $his_1^- his_2^-$ states. Or it may be shown that the $his_1^- his_2^-$ state exists by its inability to revert directly to the his^+ condition; it can revert only through another his^- state in two mutational steps. Furthermore, it is sometimes possible to separate two otherwise indistinguishable mutable units by recombination similar to that which took place in linked transformation.

Mutational as well as recombination analysis allows the identification of so-called *suppressor* (*sup*) genes. These cause phenotypes resembling those due to the wild-type state of another mutable unit. Thus what appears to be a back mutation, e.g., $his^- \rightleftharpoons his^+$, may in reality be a genotypic change in a suppressor gene, e.g., $his^- sup^- \rightleftharpoons his^- sup^+$. We therefore define the mutable unit as the smallest part of the genetic

material which may change from one condition to another in a single mutational step and which, when changed, is replicated in the changed form.

As satisfactory as this definition is conceptually, it demands much of the investigator in practice. We know from the experiments with nitrous acid that the change of a single base in the polynucleotide chain can result in a mutation; therefore, we know that the mutable unit under investigation in such experiments may be no larger than a single nucleotide. But most experiments allow no such precise statements. We may, for example, observe the following relationship in *E. coli*:



The functional gene allowing some step in the synthesis of histidine probably consists of a specific sequence of a large number of nucleotide pairs in the bacterial DNA. A change in any one of these nucleotides, or in certain critical ones, may change the gene so that it can no longer operate in histidine formation. The rate of mutation of $\text{his}^+ \rightleftharpoons \text{his}^-$ would then be the sum of the rates of change of many mutable units because all his^- phenotypes seem alike. But a particular his^- mutant may have only one inappropriate nucleotide pair; the rate of back mutation from his^- to his^+ would then be the rate with which that single nucleotide pair changed to the one needed to make the gene functional again. Therefore we would be observing in one case the behavior of many mutable units and in the other case the behavior of only one.

Phenotypic changes resulting from alterations within the gene itself are referred to as *point mutations*. On the other hand, some variations in phenotype are shown to be caused by rearrangements or loss of large segments of the genetic material. But mutations observed only as heritable changes in phenotype have many properties in common. We will now take up some of these properties and leave for later chapters further consideration of the intimate nature of the change in the genetic material.

THE RANDOMNESS OF NATURAL MUTATION

We have noted a kind of unpredictability about the mutations induced by nitrous acid. Geneticists have long noticed a similar fact about natural (spontaneous) mutation, and have concluded that it is a

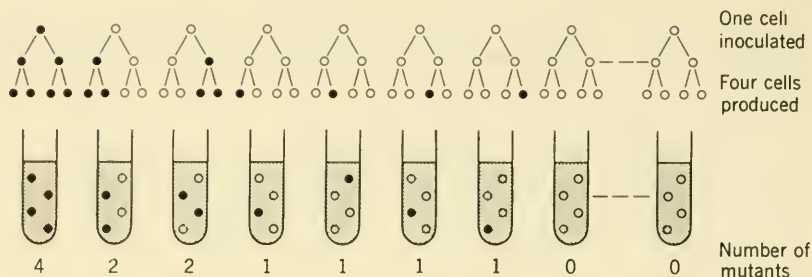


FIGURE 2.3. Diagrammatic representation of the numbers of mutants found in independent but similar clones following a single mutation in each.

random process. The notion of randomness has several aspects which may be tested experimentally. First, there is the concept of adaptive randomness. Do mutations occur without any adaptive relation to the environment in which they happen? For example, do mutations from *str-s* to *str-r* occur even when streptomycin is absent from the environment? This kind of randomness would make for an unpredictability of mutation. The statistician means something else when he speaks of a random process; he means that the chance of an event is constant with time and the same for all units in which the event may take place. We may ask whether the chance of mutation is the same for all cells in a population and constant with time. Since we know that the surroundings (e.g., nitrous acid) will change the rate, we must specify the environment in which the mutation is occurring. We may also ask whether the chance that mutation will occur in one mutable unit is independent of mutation in another. Because of the rareness of mutation, we are hard put to find experimental answers to these questions with higher forms, but relevant experiments have been performed with microorganisms.

The first rigorous test was performed by Luria and Delbrück in 1942. Using what is called *fluctuation analysis*, they took advantage of the fact that bacteria multiply by dividing in two, one cell thereby forming a clone that may consist of billions. Let us consider a number of different clones, each originating (as the definition requires) from a similar cell. For diagrammatic purposes we will consider clones of only four cells, but the principle to be demonstrated holds as well for the very large populations usually studied. Figure 2.3 shows the numbers of mutants that can be found in the various hypothetical clones. If the mutation occurs in the cell that originates the clone, all four members will be mutant; if it occurs in one of the first pair of cells formed, only two mutants will result; mutation in the final generation will yield

but a single mutant. It is clear that early occurring mutations give rise to more mutants than late occurring ones. In each case of mutation represented in Figure 2.3, only one event has occurred, yet there is a maximally broad distribution, from zero to four, of the numbers of mutants present at the end of growth. You may be struck by the fact that the number of clones with four mutants is represented as half of that with two mutants, which, in turn, is half of that with one mutant. This is because, if the chance for mutation is constant per bacterium per generation, the event is likely to occur one quarter and one half as frequently when there are one quarter or one half as many bacteria present. As a consequence, a series results in which the number of clones with two or more mutants is about half, but not quite half, of

TABLE 2.1
Agreement between the Observed Number of Clones with Different Numbers of Mutants in Phage and Bacteria and the Number Expected when Mutation is a Very Rare Event Occurring at Random in Small Populations

Phage Mutants (<i>T2</i> , <i>r</i> , and <i>w</i>) [°]		
Mutant Particles in the Phage Yield from Single Bacteria	Observed Number of Clones	Expected Number of Clones
1 or more	Total = 183	Total = 183
2 or more	90	90.8
4 or more	41	45.1
8 or more	21	22.2
16 or more	10	10.8
32 or more	4	5.1
64 or more	0	2.2

Bacterial Mutants (<i>E. coli</i> , <i>str-r</i> , and <i>str-d</i>)†			
Divisions Prior to End of Growth	Number of Descendants from a Mutation	Observed Number of Clones	Expected Number of Clones
0	1	29	21.8
1	2	10	10.9
2	3-6 (4)	1	5.4
3	7-11 (8)	2	2.7
4	12-22 (16)	0	1.4
5	23-45 (32)	<u>1</u>	<u>0.7</u>
		Total = 43	Total = 42.9

[°] From Luria, 1951, *C.S.H. Symp. Quant. Biol.*, **16**:463.
[†] From Newcombe and Hawirko, 1949, *J. Bact.*, **57**:565.

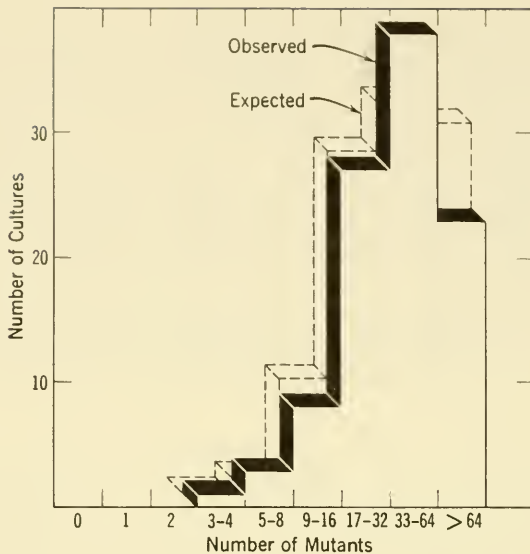


FIGURE 2.4. Actual distribution of the numbers of lactose fermenting mutants of *E. coli*. in different cultures of nonfermenters compared with the theoretical distribution calculated according to Lea and Coulson. The probability of drawing a sample like that observed from a population of cultures in which mutant numbers were distributed according to the theory is 0.2 (after Ryan, 1952, *Nature*, 169:882).

the number with one or more mutants, and so on. In Figure 2.3 the number with one or more is seven; with two or more, three; and with four or more, one. As the maximum size of the mutant clone is increased, there is a closer approach to one half. This pattern has been observed for both bacteria and bacterial viruses (Table 2.1).

In these considerations, the assumption has been made that mutation is a very rare event so that the overwhelming majority of cultures have none. Only when they are rare is it likely that two or more mutations will not occur in the same clone. In case they did, one could not be sure, by observing only the final number of four mutants, whether they arose by four mutations in the last generation, two mutations one generation back, or one mutation two generations back. Clearly, as the new mutants grow, mutations and mutants no longer equal one another in number. In usual practice, although the chance of mutation is small, the number of bacteria is so large that the occurrence of several mutations in a single large clone is a realizable event. Luria and Delbrück predicted and observed the great variation in numbers of mutants among different cultures (Table 2.2), and Lea and Coulson in England gave a

general solution to the difficult mathematics of the actual distribution. In some cases, the distributions observed fit very well the unique distribution predicted on the assumption that mutations occur during growth at a constant rate (Figure 2.4). These experiments provide evidence for the statistical randomness of mutation.

Statistical Randomness

The statistical randomness of mutation, or the constancy of the chance that it will occur, has two components, one of time and one of space. Lea and Coulson assumed for their calculations that this chance was the same at all periods during growth and for all bacteria in the populations.

TABLE 2.2

Number of Streptomycin-Resistant *E. coli* in Samples from 20 Independent Cultures and from 15 Similar Samples from a Single Culture
(From Demerec, 1948, *J. Bact.*, 56:63)

Experimental Samples from Independent Cultures				Control Samples from a Single Culture			
Culture Number	Number of Resistant Bacteria	Culture Number	Number of Resistant Bacteria	Culture Number	Number of Resistant Bacteria	Culture Number	Number of Resistant Bacteria
1	67	11	56	1	142	11	110
2	159	12	91	2	155	12	125
3	135	13	123	3	132	13	135
4	291	14	97	4	123	14	121
5	75	15	48	5	140	15	112
6	117	16	52	6	146		
7	73	17	54	7	141		
8	129	18	89	8	137		
9	86	19	111	9	128		
10	101	20	164	10	121		
Average			105.9				131.2
Variance°			2913.9				151.1
χ^2			550.3				17.3
Probability of drawing a sample with a χ^2 as large or larger from a population of cultures in which the mutant numbers were distributed at random			0.001				0.26

°The sum of the differences from the mean (average) squared and divided by the number of entries minus one [$\Sigma d^2 / (n - 1) = \text{standard deviation}^2 = \sigma^2$].

As a consequence, because the distribution they predicted was observed, we cannot reject either kind of randomness as a property of mutation. These two components of randomness can be examined separately. For example, when the mutation rate is measured after different periods of growth of bacteria under identical conditions, it is always found to be the same. The existence of the same chance for all, or at least most, of the bacteria has been demonstrated in another way. The method employed uses the Poisson distribution, which must first be described.

Consider, as an example, a gentle spring shower in which the raindrops, affected by so many opposite forces during their formation and fall to earth, are truly distributed at random. Suppose we were to rule a sheet of paper into 100 squares of equal size and expose it to this shower until exactly 100 hits had been registered; the average number of hits per square would be 1, but it is a matter of experience that each square will not have been hit by one raindrop. Some squares will not have been hit at all, some will have been hit once, some twice, etc. Few squares will have many hits, of course, and the average number of squares hit not at all, once, twice, etc., is approximated by the Poisson formulation. This is a special case of the binomial theorem which holds when the actual number of events that does occur is insignificantly small compared to the number the system could entertain. Mathematically, it is written

$$P_{(x)} = \frac{m^x}{x!} e^{-m} \quad (2.1)$$

where $P_{(x)}$ is the chance that x events will occur, $x!$ is x factorial (e.g., $3! = 1 \times 2 \times 3$), m is the average number of events per unit, and e is 2.718, the base of natural logarithms. $P_{(x)}$ is, of course, approximated by the observed frequency with which x events occur per unit, and in this way the equation will pretty well describe the distribution of numbers of raindrops hitting the squares on the paper at random.

This formulation has a usefulness in biology in general, and especially in genetics. Much of biology, in contrast to classical chemistry, is concerned with units, i.e., with discontinuities. Chemists are usually interested in the average behavior of billions upon billions of molecules and can express rates of reaction by differential equations. The biologist, like the physicist studying atomic particles, usually deals with discrete units in small numbers, whether they be organisms, cells, microscopically visible subcellular particles, or operationally defined units. His subject matter is so often particulate that the mathematics he employs is frequently the statistics of counting. Furthermore, his chemistry, so often

dealing with macromolecules which may sometimes be present only once per cell, has no precedent.

The Poisson formulation ought then to predict the numbers of mutations occurring among different clones of cells if they take place at random. It is important here to distinguish numbers of mutations from numbers of mutants which we saw were distributed among different clones according to another formulation, that of Lea and Coulson.

It is possible to make a rather direct estimate of the distribution of numbers of mutations among different clones by using a solid culture medium instead of liquid for growing the cells, and thereby counting mutant clones instead of mutant cells. When bacteria which cannot use lactose are grown on an amino acid medium, mutants appear which can use that sugar. On the surface of agar containing amino acids and lactose, the nonutilizing parents form a colony; the mutants arising within it, by also using the lactose present, overgrow to form papillae on the colony surface. If an indicator dye is incorporated in the medium, the lactose-utilizing papillae turn red because they form certain specific waste products. On the other hand, the colony background is almost white, allowing a ready enumeration of the papillae. Here each colony is a separate culture and each papilla represents one mutation, irrespective of the number of mutant bacteria it contains. In this way the mutations themselves may be counted, even though a constant fraction may not express them as papillae. If almost all the parental lactose-nonutilizing bacteria have the same chance of mutating per division, then a Poisson distribution of the numbers of papillae (but not, of course, of the number of mutant cells composing them) should be observed. The numbers of colonies with no mutations, or with one, two, or more, are shown in Figure 2.5, along with the Poisson distribution expected for the average number of mutations observed. The close similarity of the two distributions indicates that the mutations were randomly distributed among the bacteria making up the various colonies.

Still one more component of statistical randomness exists; it involves independence in the mutation of one gene from mutation in another. This criterion seems to be met by mutation in plants and animals, but it can be shown most clearly to govern mutation in bacteria. Double auxotrophic mutants can be obtained which have changes in two different genes, each one causing a requirement for a different substance needed for growth. For example, mutant bacteria requiring both the amino acids histidine and methionine have been obtained in two steps. First the strain became histidineless (histidine-requiring, or *his*⁻) by one mutation; then a second mutation occurred which gave rise to a histidineless-methionineless type (*his*⁻ *met*⁻). This double auxotroph back-

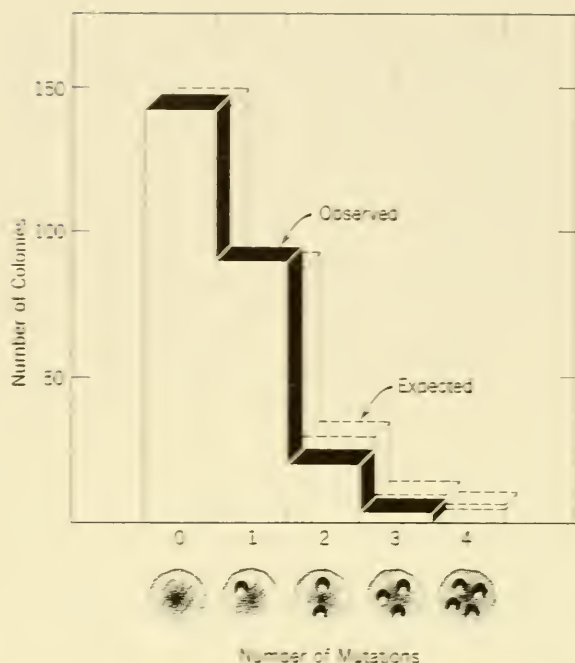


FIGURE 2.5. Actual distribution of numbers of lactose-utilizing mutations in different colonies of *E. coli* compared with the Poisson distribution. The probability of drawing a sample like that observed from a population of colonies in which the mutations were distributed according to theory is 0.2 (Ryan, unpublished).

mutates to conditions where it is either only methionineless (*his⁻ met⁻*) or histidineless (*his⁻ met⁺*) but it is never observed to back-mutate in one step to the original nonrequiring, *prototrophic* state (*his⁺ met⁺*). This suggests that mutation in the two genetic units is not a linked process but that one mutable unit can mutate independently of the other: the chance that the two will mutate in the same cell is the very small product of the chances of mutation in the independent genes (e.g., $10^{-5} \times 10^{-5} = 10^{-10}$). Furthermore, when the number of mutants of the two sorts is determined in many different cultures, no correlation is found (Figure 2.6). We may conclude, therefore, that mutation in the gene determining the histidineless condition is independent of mutation in the gene for the methionineless state, and, once again, that most of the cells are equally mutable. This free behavior in mutation has been found for most genes studied; the status of some apparent exceptions is uncertain because of alternative structural and physiological possibilities. Independence in mutation demonstrates that stability is an independent

property of each mutable unit: these units are the individual members of the genotype.

Adaptive randomness

In most of the experiments so far described, the mutants have been selected on a special medium. For example, *str-r* mutants are identifiable only in the presence of streptomycin. But we could suppose that in the course of testing for mutants the streptomycin itself induced the change. How then do we know that the *str-r* mutants arose during previous growth in the absence of streptomycin?

The fluctuation analysis and the distribution of numbers of mutants in different cultures answer this question for us. Were there a small chance that each cell exposed to streptomycin would mutate, the distribution of numbers of mutants in different cultures should follow the Poisson formula. It is a property of this distribution that the variance (σ^2) equals the average. We have seen that the variance of observed numbers of mutants is many times the mean (Table 2.2); the distribution is not Poisson. Therefore we must reject the hypothesis that the mutations were induced by streptomycin; instead they occurred during the growth in the absence of streptomycin which preceded exposure.

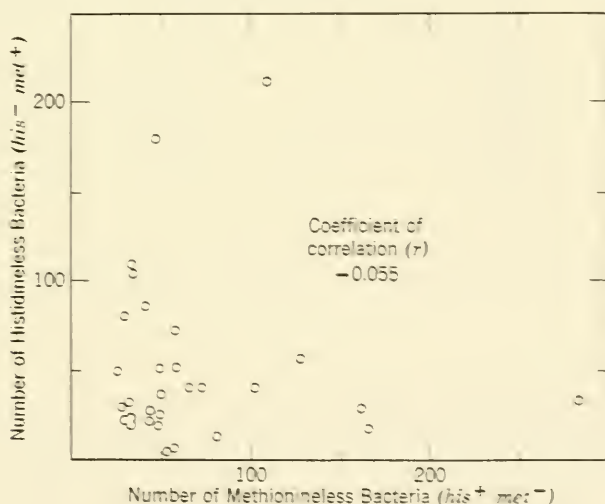


FIGURE 2.6. Comparison of the number of single mutations of two sorts occurring in a double auxotroph of *E. coli* that is both histidineless and methionineless (*his*⁻ *met*⁻) (Ryan, unpublished).

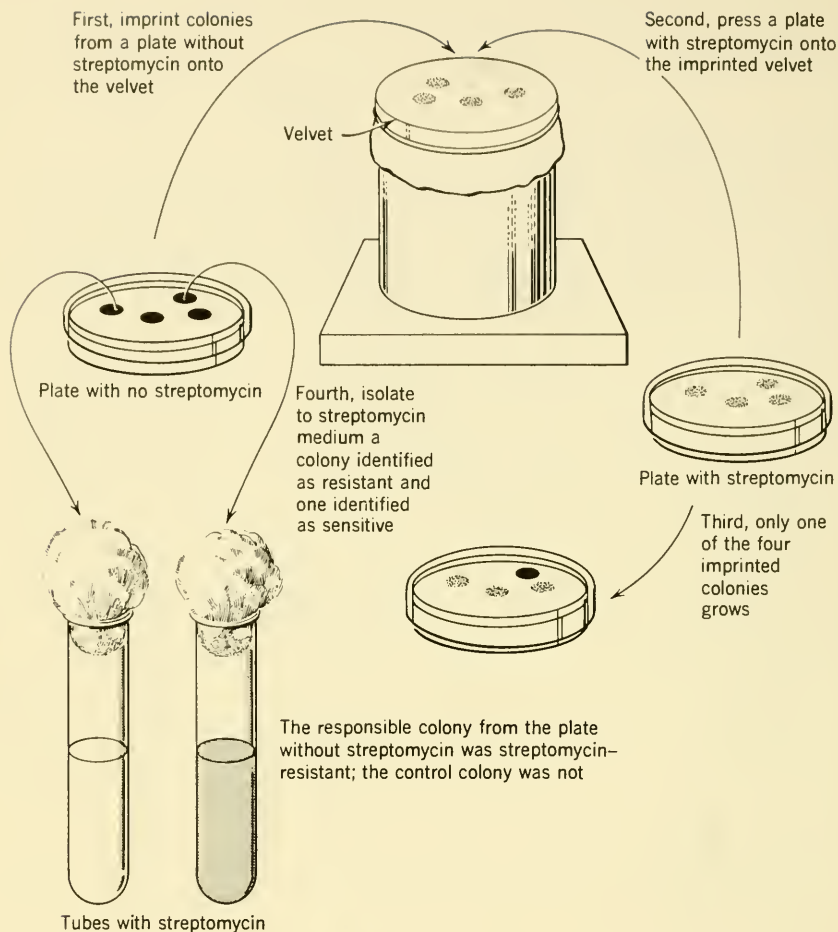


FIGURE 2.7. The replica-plating technique which demonstrates the preadaptive nature of mutation. For diagrammatic reasons, too few colonies are indicated on the plates.

Another test of this issue was devised by the Lederbergs. They took advantage of the fact that when a piece of sterile velvet is pressed gently against an agar plate the fine hairs of the fabric pick up some cells from each colony present. In this way many colonies grown on medium free of streptomycin, for example, can be imprinted on velvet, from which an identical print can be made onto agar containing streptomycin. This method, diagrammatically shown in Figure 2.7 is called

replica plating. On the streptomycin agar, whole colonies form only in the spot where, on the original plate, a colony of streptomycin-resistant bacteria existed. This resistant colony can be identified by comparing the plates; when it is isolated and tested, it is proved to have been streptomycin-resistant even though it had never encountered that drug. This proves that the mutation occurred in the absence of the selective agent; i.e., it was preadaptive.

Nonetheless, certain apparently nonchromosomal mutations are specifically induced by chemical agents. They will be discussed in Chapter 9.

MUTATION RATES

Good estimates can be made of the magnitude of the rates (chances) of mutation. What is measured is the average frequency with which the mutational event takes place per cell per division. What is estimated is the frequency of mutation in an infinite number of cell divisions. Since the event is random, this is the chance of its occurrence. Among the units that define this chance is found the term "per division," which introduces a time element and makes the chance a rate. Of course, as we have already seen, the frequency of mutation is necessarily different from the frequency of mutants which is often what is measured. There are several methods of estimating the mutation rate from the number of mutants.

Another, simpler method does not depend on the number of mutants but rather upon the frequency of clones that contain no mutants. This method employs the Poisson distribution (equation 2.1) which can be simplified when $x = 0$, for then m^x and $x!$ both equal one. We may therefore write:

$$P_{(0)} = e^{-m} \quad (2.2)$$

which is readily solved in this form:

$$\ln P_{(0)} = -m \quad (2.3)$$

where the symbol $\ln P_{(0)}$ stands for the natural logarithm (to the base e) of $P_{(0)}$. In our example of the paper exposed to the shower, we knew that the average number of raindrop hits per square was one. We can therefore quickly calculate that $P_{(0)} = 0.37$. Thus about 37 per cent of the squares would not have been hit at all. Conversely, if we knew only $P_{(0)}$, we could easily calculate m .

The latter is more like the case with mutation where we can measure

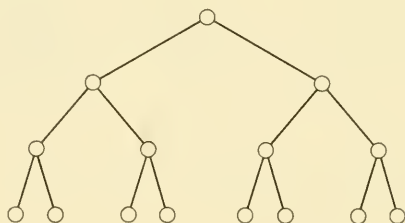
$P_{(0)}$ as the *fraction* of cultures which had no mutational events, and from this calculate m . The assumption is that the mutational events are distributed at random, and this has been amply demonstrated to be the case. Experimentally, $P_{(0)}$ may be measured by plating cultures of bacteria on agar media where mutants may be detected. The frequency of mutants is usually so small (ea. 10^{-7}) that the medium preferably should be selective, allowing no growth of the parents. Thus, among the many millions of the latter on the plate, only the mutants present will form colonies.

This method of measuring mutations has been adapted for use with liquid cultures and also is conveniently employed in cases where mutants form papillae on the surface of colonies. In this case some colonies may have no papillae at all. Letting this fraction equal $P_{(0)}$, the average number of papillae, m , can be calculated from equation 2.3. Since each papilla represents one mutational event, the calculated m can be compared with the m obtained by counting the number of papillae directly. The two estimates are found to agree because mutation is random.

Once m is known, the chance of mutation per cell per division, α , can be estimated from the equation defining mutation rate:

$$\alpha = m/d \quad (2.4)$$

where d is the total number of divisions that have taken place. The term d cannot be measured directly but it is in some cases approximately equal to N , the total number of cells present per culture. This can be seen by considering the properties of a clone produced by repeated division into two:



Here one division gave rise to two, three divisions to four, and seven divisions to eight cells. The number of divisions counted from the first cell is one less than the number of cells produced. As a consequence, when a large culture is started from a few cells, $d \approx N$, and N may be substituted for d in equation 2.4. In this way one can measure the rate of mutation of many microorganisms which grow as separate cells; the method can be adapted for the somatic cells of higher organisms as well.

Table 2.3 shows a sample of such mutation rates. They range in bacteria, for example, from 10^{-6} to 10^{-9} per cell per division. But we realize that these rates are simply the chance with which the phenotypic change is observed. Even if all these mutations were point mutations, we would not know in any particular case whether one or many mutable units were involved. Therefore we expect variation in the

TABLE 2.3
A Sample of Spontaneous Mutation Rates in Different Organisms

Organism	Character	Rate	Units
Bacteriophage: <i>T2</i>	lysis inhibition, $r \rightarrow r^+$ host range, $h^+ \rightarrow h$	1×10^{-8} 3×10^{-9}	per gene* per replication
Bacteria: <i>Escherichia coli</i>	lactose fermentation, $lac^- \rightarrow lac^+$ phage <i>T1</i> sensitivity, $T1-s \rightarrow T1-r$ histidine requirement, $his^- \rightarrow his^+$ $his^+ \rightarrow his^-$ streptomycin sensitivity, $str-s \rightarrow str-d$ $str-d \rightarrow str-s$	2×10^{-7} 2×10^{-8} 4×10^{-8} 2×10^{-6} 1×10^{-9} 1×10^{-8}	per cell per division
Algae: <i>Chlamydomonas reinhardi</i>	streptomycin sensitivity, $str-s \rightarrow str-r$	1×10^{-6}	
Fungi: <i>Neurospora crassa</i>	inositol requirement, $inos^- \rightarrow inos^+$ adenine requirement, $ade^- \rightarrow ade^+$	8×10^{-8} 4×10^{-8}	mutant frequency among asexual spores
Corn: <i>Zea mays</i>	shrunken seeds, $Sh \rightarrow sh$ purple, $P \rightarrow p$	1×10^{-5} 1×10^{-6}	
Fruit fly: <i>Drosophila melanogaster</i>	yellow body, $Y \rightarrow y$, in males $Y \rightarrow y$, in females white eye, $W \rightarrow w$ brown eye, $Bu \rightarrow bu$	1×10^{-4} 1×10^{-5} 4×10^{-5} 3×10^{-5}	mutant frequency per gamete per sexual generation
Mouse: <i>Mus musculus</i>	piebald coat color, $S \rightarrow s$ dilute coat color, $D \rightarrow d$	3×10^{-5} 3×10^{-5}	
Man: <i>Homo sapiens</i>	normal $\rightarrow$ hemophilic normal $\rightarrow$ albino	3×10^{-5} 3×10^{-5}	per cell per division
Human bone marrow cells in tissue culture	normal $\rightarrow$ 8-azoguanine resistant normal $\rightarrow$ 8-azoguanosine resistant	7×10^{-4} 1×10^{-6}	

* Correction of the other mutation rates in this table to a per-gene basis would not change their order of magnitude.

rates of mutation. In multicellular organisms, these rates vary from gene to gene, but together they form a class which averages about 10^{-5} . They are not to be compared directly with the rates of mutation in microorganisms which are true rates; in intact higher organisms, the so-called rates of mutation are only the frequencies of mutant gametes expressed as per generation. As such they do not directly reflect the mutation rate because clones of mutant cells result from one mutation. As the mutant cells divide and new mutations occur, the frequency of mutants continues to increase. Thus older organisms have more mutant gametes than younger ones. But even when the true rates of mutation of mammalian somatic cells are estimated by enumerating papillae in tissue culture, they are found to be high.

It is possible that the absolute rates of mutation, or at least mutant frequencies, are, in general, selected in the course of evolution to be those maintaining the optimal proportion of mutants in the population. Sexually reproducing higher organisms, for reasons of population genetics, may maintain greater mutational loads than microorganisms. The question of what constitutes an optimal mutational load is an important one, especially now that man is increasing the level of radiation in his environment, but it will not be discussed further at this point.

The background of other genes, and probably of other kinds of hereditary determinants, controls the stability of a given gene. An example is the case of mutation to yellow body color in the fruit fly where the rate differs significantly in male and female. Similar examples exist in which strain, not sex, is responsible. A most interesting case is that in which one single gene determines the rate of mutation of another. Such *mutator genes* are known in plants and animals, and in microorganisms. Some are nonspecific and cause many different genes to increase their mutation rates. In the corn plant, however, there is one which shows a high degree of specificity; the mutator gene, *Dt*, increases only the rate of mutation of gene *a* to its allele, *A*. All cells containing the *A* gene form a purple pigment. It is interesting that gene *Dt* acts only at a specific time in the formation of the kernel. We know this because on the kernel surface there are purple patches of the same size; each contains about the same number of cells, all derived from the one which originally mutated (Figure 2.8). This is a case of the induction of a specific mutation, apparently random in space but nonrandom in time. Because it involves the influence of one gene on another, a specific chemical mutagen is implicated but its nature is unknown. In other cases, a gene may be unstable and mutate with a rate as high as 10^{-2} per division, not because of other genes but perhaps for intrinsic reasons.

Still other genes have not been observed to mutate at all; some of them

are cases of *deletion* where the actual substance of a gene may be absent. Occasionally, deletions of parts of chromosomes may be so large as to be visible with the microscope. Unless another complete chromosome is also present in the same cell, large deletions are usually lethal, probably because they include at least one gene essential for the life of the cell. But not all lethals are deletions. This histidine-requiring mutant referred to in Table 2.3 is a lethal in an environment not containing that amino acid; the fact that it back-mutates shows it is not a deletion. Lethals may be maintained for study either if there are other genes in the cell which can perform the same function or if the cell can otherwise be supplied with the needed requirement. Mutation to lethal conditions has provided methods for measuring mutation rates that are not influenced by the subjective errors involved in scoring visible traits.

CONCLUSION

The properties of the mutable unit described in this chapter are drawn largely from examples of mutation in bacteria. As stated at the outset, bacterial populations provide convenient systems for the analysis of this process, but the legitimate question may be asked whether the results found among them can be generalized. Certainly nothing is known from studies of higher organisms or of other microorganisms that contradicts the findings in bacteria. It is perfectly reasonable to expect that in the course of evolution the genetic systems of bacteria and other organisms have come to differ. But there also are common denominators—for example, the nucleotides they contain. Recent work in biochemistry and genetics has greatly strengthened our evidence for the basic unity of

FIGURE 2.8. A kernel of maize possessing the genes α and Dt . The fact that the spots are small and of approximately equal size shows that the mutations from $\alpha \rightarrow A$ induced by the Dt gene all occurred at about the same time late in development (after Rhoades, 1936, *J. Genetics*, 33:347).



life processes. The pre-eminent position that genes occupy in the economy of the cell justifies the presumption that they are universally similar.

A more serious limitation remains, however, in our attempts to study mutation in any cell or any organism. In experiments on the mutable unit what is observed is the mutant organism. The new genetic constitution is seen through the filter of all the processes which intervene between it and the cell character it determines. This may cloud our vision of the gene. For example, would genes that did not mutate, or that were so unstable as to pass unobserved back to the original state, be different in structure from others? Again, the mutant units studied are those which influence cell characters to such an extent and in such a way that they can be detected and analyzed. Is this selected class unrepresentative? There are reparable and irreparable lethal mutations. The former can be easily investigated, the latter with difficulty. Are genetic units with irreparable functions different from others? Even if they mutate in the same fashion because of similarities in structure, do they function differently? May they, for example, have more than one function? These questions have been investigated, although insufficiently. The probable answer to all of them is "no." Although we accept this answer with reservation for the reasons enumerated, there seems to be a basic similarity in all genes. The similarity in mutation may be greater because the mutable unit is only a small part of the segment of nucleic acid which acts as a functional genetic unit. These small parts, the nucleotides, may be the common denominators, functional differentiation occurring at higher levels of organization.

What image of the gene can be created from the facts thus far presented? In the first chapter we saw that in chemical composition genes are probably nucleic acids; in many cases, at least, DNA. They are the residence of the information necessary for the metabolism and development of the cell. Thus they form the thread of life extending from generation to generation.

Considerations from mutation analysis lead us to conclude further that genes are probably:

1. Particulate, at least in behavior; one of many small units making up the genotype;
2. structures of considerable stability but subject to rare spontaneous and induced change back and forth among a variety of possible states;
3. subject to a mutation that is random in space and time, that usually occurs independent of mutation in other genetic units, and that has no adaptive relation to the environment in which it takes place;

4. subdivisible, inasmuch as the mutable unit may be as small as nucleotides whereas the gene need contain many nucleotides to possess the specificity necessary for function.

These are the properties of a macromolecule as revealed through the study of mutation by biological methods, all the more remarkable because a given gene is represented by at most a few copies in each cell. Mutations have a chemical mechanism, and the genetic material in its alternative states controls the character of the cell by influencing its biochemical behavior. These subjects will be dealt with in later chapters. It is convenient first to continue our considerations of the structure of genes and to turn to another biological method which studies the recombinations that occur among them.

BIBLIOGRAPHY

- Auerbach, Ch., 1949. Chemical mutagenesis. *Biol. Reviews*, **24**:355-391.
- Brookhaven Symposia in Biology No. 8, 1956. *Mutation*. Washington, Office of Technical Services, U. S. Dept of Commerce, 231 pp.
- Cavalli-Sforza, L. L., and J. Lederberg, 1953. Genetics of resistance to bacterial inhibitors. *Symp. on Growth Inhibition and Chemotherapy*. Rome, Istituto Superiore di Sanita, pp. 108-142.
- Cavalli-Sforza, L. L., 1957. Indirect selection and origin of resistance. In: G. E. W. Wolstenholme, and C. M. O'Connor, Ciba Foundation, *Symp. on Drug Resistance in Microorganisms*. Boston, Little, Brown & Co., pp. 40-46.
- Hershey, A. D., 1946. Spontaneous mutations in bacterial viruses. *C.S.H. Symp. Quant. Biol.*, **11**:67-77.
- Lea, D. E., and C. A. Coulson, 1949. The distribution of the numbers of mutants in bacterial populations. *J. Genetics*, **49**:264-285.
- Lederberg, J., 1951. Inheritance, variation and adaptation. In: Werkman, C. H. and P. W. Wilson, *Bacterial Physiology*. New York, Academic Press, pp. 67-100.
- Lederberg, J., and E. Lederberg, 1952. Replica plating and indirect selection of bacterial mutants. *J. Bact.*, **63**:399-406.
- Luria, S. E., and M. Delbrück, 1943. Mutations of bacteria from virus sensitivity to virus resistance. *Genetics*, **28**:491-511.
- Muller, H. J., 1928. The problem of genetic modification. *Z. indukt. Abstammungs- u. Vererbungslehre*, Suppl. **1**:234-260 (Verh. V Intern. Kongr. Vererbungswiss).
- Schuster, H., 1960. The ribonucleic acids of viruses. In: E. Chargaff and J. N. Davidson, *The Nucleic Acids*, Vol. III. New York, Academic Press. pp. 245-301.
- Timofeeff-Ressovsky, N. W., 1934. The experimental production of mutations. *Biol. Reviews*, **9**:411-457.

3

Recombination in Sexual Organisms

The genius of Gregor Mendel's insight into heredity lies in the fact that he conceived of it as discontinuous. While working with the garden pea, he decided to focus his attention on one difference at a time, such as that between pink and white blossoms. Putting aside for the moment all other differences, he counted the numbers of each type of offspring from the crosses he made and avoided the trap that caught his forebears. They had tried to consider all characters at once and could not see the simple laws that governed the inheritance of something as complex as an individual. Mendel, on the contrary, postulated the existence of unit factors and gave an abstract solution to his problem. The rules which he established for the behavior of unit factors accounted in a formal way for what he saw and provided a basis for predicting the results of future experiments.

In this chapter we will consider the transmission of hereditary factors in sexual reproduction, starting not with the pea plant, as Mendel did, but with some microorganisms which illustrate basic principles more clearly. Subsequently, we will see how the same laws govern the genetic behavior of higher organisms.

SEGREGATION AND ASSORTMENT

First, let us consider inheritance in the green alga, *Chlamydomonas*. There are two "sexes," or more properly, two *mating types*, referred to as mt^+ and mt^- because we cannot see any morphological difference between them. But they know the difference; mt^+ individuals will choose only mt^- cells as their mates, and *vice versa*. When they mate, as shown

in Figure 3.1, their bodies actually fuse together in a process called *syngamy*, comparable to the fertilization of an egg by a sperm. This results in the formation of a zygote which, in time, gives rise to four gametes. Each of these divides asexually to produce large clones of identical cells. Half of the clones are of mt^+ and half of mt^- mating type. Under proper conditions, individual mt^+ and mt^- cells may fuse to form a zygote once again, and the process repeats.

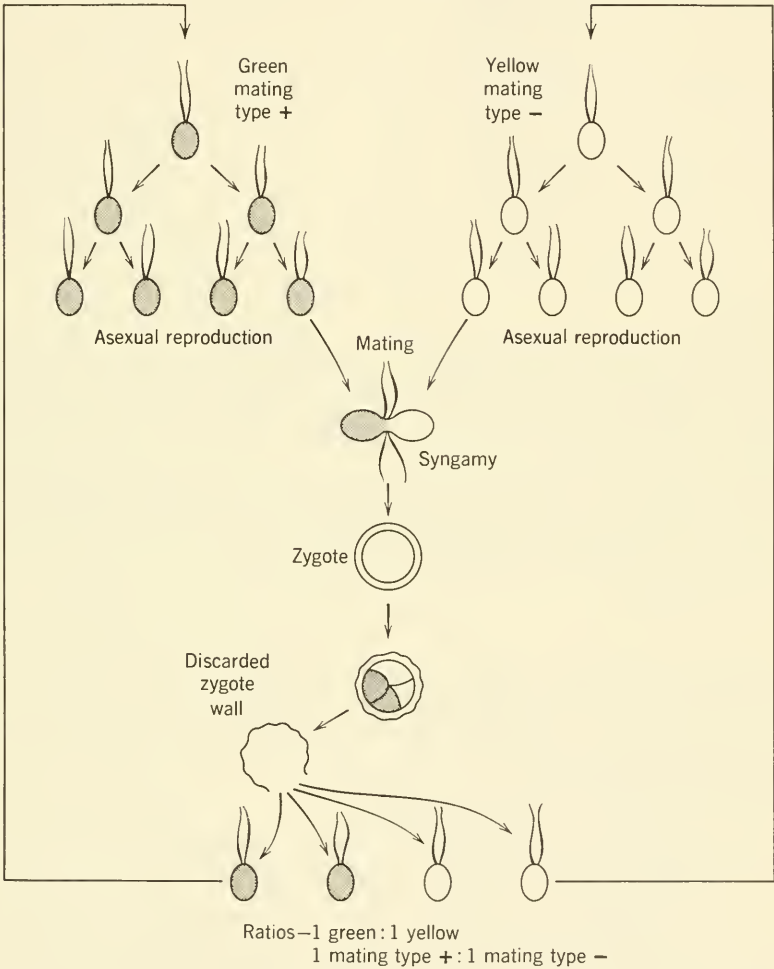


FIGURE 3.1. Diagram of the life cycle and the segregation of color in the unicellular green alga, *Chlamydomonas reinhardtii*. This microscopic fresh-water inhabitant swims by using its whiplike flagellae. The normal green type contains chlorophyll and can live by photosynthesis.

In the course of asexual reproduction, mutations may occur giving rise, for example, to colorless mutants which lack chlorophyll and can grow on sodium acetate without photosynthesis. When a normal green strain is mated with the colorless mutant of opposite mating type, a very simple result is obtained. Each zygote gives rise to two green and two colorless offspring. This 1:1 ratio is almost invariant and indicates that the hereditary determinants for greenness pass through the zygote unimpaired. Pairs of genes of this sort, called *alleles*, segregate from one another in the formation of gametes. This is Mendel's first law.

We can think of other ways in which the unit factors could behave when together in the zygote. They might interact by altering one another—but they do not. They retain their identity and segregate inviolate into the four daughter gametes. They might be distributed at random into these cells. Thus, if only a few factors of each kind were in the zygote, some of the resulting gametes would have none. But this is not the case. Rather, a regular mechanism of segregation insures that each daughter cell will receive one parental factor, either the paternal or the maternal one.

Mendel's second law states that the segregation of one pair of alleles is independent of the segregation of a second pair. Thus if the letters *A* and *a* represent one pair, and *B* and *b* another, we may write the genotypes of the parents in a cross as *AB* × *ab*. Half of the gametes will receive *A* and half *a*; half of the gametes will receive *B* and half *b*. If the possession of a specific member of the *A/a* set has no influence on which of the *B/b* set will be present, then one quarter ($0.5 \times 0.5 = 0.25$) will have each of the four possible combinations:

$$(\frac{1}{2}A + \frac{1}{2}a) \times (\frac{1}{2}B + \frac{1}{2}b) = \frac{1}{4}AB + \frac{1}{4}Ab + \frac{1}{4}aB + \frac{1}{4}ab$$

In *Chlamydomonas* the factor pairs for color (green = y^+ , yellow = y^-) and for mating type (*mt*) segregate independently. For example, from a cross of $y^+ mt^+ \times y^- mt^-$ 183 gametes were collected at random. Their genotypes were as follows:

$y^+ mt^+$	43	} 87	Parental types
$y^- mt^-$	44		
$y^+ mt^-$	54	} 96	Recombination types
$y^- mt^+$	42		
Total	183		

The numbers of different types obtained are approximately equal to the 45.75 of each anticipated if they were formed with a frequency of

TABLE 3.1

A Statistical Test by the Method of Chi Square (χ^2) of the Significance of the Differences between the Numbers of Randomly Sampled Gametes Containing Recombinations of Genes and the Numbers Expected on the Hypothesis of Random Assortment

Parental Genotypes: $y^+ mt^+ \times y^- mt^-$	Parental-Type Gametes	Recombination Gametes
Observed number	87	96
Expected number	91.5	91.5
Difference	4.5	4.5
Difference corrected	4	4
$(\text{Difference corrected})^2$	16	16
$\frac{(\text{Difference corrected})^2}{\text{Expected number}}$	0.18	0.18
Sum $\frac{(\text{difference corrected})^2}{\text{expected number}} = \chi^2 = 0.36$		
$n = 1, P = 0.55$		

Many textbooks of statistics contain tables relating χ^2 to P for any n . The number of degrees of freedom, n , is here one less than the number of classes observed (two). Once we know the total number of members (183) in the two classes and the number in any one class, then the number in the other class cannot vary—it must give the total when added to the first. P is the probability of obtaining χ^2 as large or larger than a given value when the statistical hypothesis being tested is true and the deviations are due to random sampling error. About 55 per cent of samples, drawn from a population where old and new combinations were actually present in a 50:50 ratio, deviate as much from expected as this sample did. It is likely, therefore, that the discrepancy is due to sampling. It is concluded that the data are consistent with the hypothesis of random assortment and that the genes segregate independently.

A P of 0.05 is usually considered critical; if random sampling alone so infrequently yielded such deviations, the hypothesis would be suspect. It should be noted that numbers, not frequencies, were used for the calculations; this is because the sensitivity of the χ^2 test depends upon the absolute size of the numbers employed. The Yates' correction made of the differences consists in bringing them one half unit closer to zero. It is imperative to use this correction when the numbers being tested are small. The example given above is a simple application of the χ^2 test; it may be adapted to the test of more complicated comparisons but not without the understanding of the χ^2 method that can be obtained from a textbook of statistics.

The system being tested must also be understood. For example, the numbers of new and old combinations among the gametes produced in the same meioses cannot be employed as above in χ^2 test. Once a crossover event has occurred in meiosis, the genotypes of all the progeny spores are fixed. Thus, the genotype of one gamete was not obtained independently of its three sisters. Special methods must be used for such tetrad analysis.

exactly 0.25. It will also be observed that the frequency of parental types is approximately equal to the frequency of recombinants. Thus when there is 50 per cent recombination in a cross, we may conclude that the factor pairs have assorted independently. No sample of data of this sort is likely to give an exact correspondence with theory because chance enters into each combination, and the average result tends to equal the theoretical as the sample size increases. Table 3.1 shows how such data are objectively assessed.

LINKAGE, RECOMBINATION, AND GENETIC MAPS

Independent assortment is frequently observed but, unknown to Mendel, it is far from universal. Exceptions were first discovered by Bateson and Punnett in England; they referred to nonindependent assortment as *linkage*. In *Chlamydomonas* the factors determining a requirement for the amino acid, arginine (*arg*), and the vitamin, para-aminobenzoic acid (*paba*), are linked. In a cross of $arg^+ paba^+ \times arg^- paba^-$ strains, 5214 gametes were found to be old combinations while only 906 were new. This is a significant deviation from the 3060 of each type expected if the factors had assorted at random. There is a tendency for the factors *arg* and *paba* to enter the progeny in the same association as existed in the parents. When the cross was made with the opposite association, $arg^- paba^+ \times arg^+ paba^-$, the parental combinations again predominated, 618 to 146. The frequency of recombinations was about the same in the two crosses, 0.19 in the first and 0.15 in the second. It is, therefore, not the particular combination of factors which determines whether it will be present in excess, but rather the coupling with which the factors entered the cross.

The strains used in the crosses just mentioned differed in another trait, the requirement for the vitamin, thiamin (*thi*), so that the genotypes in the crosses may be rewritten:

$$\begin{array}{l} arg^+ paba^+ thi^- \times arg^- paba^- thi^+ \\ arg^- paba^+ thi^+ \times arg^+ paba^- thi^- \end{array}$$

The genotypes of the progeny of these three-factor crosses show that *thi* is also linked to *paba*. Among the 6884 gametes analyzed, 30 per cent showed new combinations of these genes and, once again, this frequency was independent of the coupling in which they entered the cross. If, as has been shown, *arg* is linked to *paba* and *paba* is linked to *thi*, then *arg*

must also be linked to *thi*. When the 6884 gametes studied in these crosses were examined for the combinations of *arg* and *thi* genes, it was found that 43 per cent contained new combinations. This frequency, because of the large number of gametes studied, is also significantly different from 50 per cent, so we must conclude that *arg* and *thi* are also linked, even if less strongly. Thus we know that *arg*, *paba*, and *thi* are linked and tend to pass into the gametes in parental association, whatever that association may be in a particular cross.

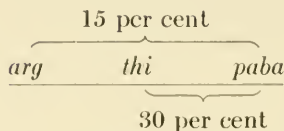
In addition to this case of linkage in *Chlamydomonas*, we have already seen another in pneumococcus. The factors *str-r* and *mtl* showed linkage in transformation. In the genetic studies of all organisms, it is found that some factor pairs recombine freely whereas others undergo restricted recombination yielding recombinants in frequencies varying from 0.0 to 0.5, the actual value being characteristic of the allelic pairs.

The fact of linkage suggests that genes are arranged as parts of some cell structure. Because recombination takes place, linkage is not complete; exchange of genetic material occurs with a frequency that must be a function of the particular physical association between two given genes. In a purely formal way, we can draw some conclusions about this physical association, using the results of recombination alone. For example, it is possible to show that genes are arranged in a linear order and to make a map of their sequence.

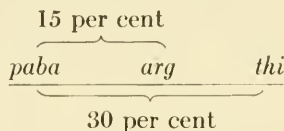
The principle is very simple. We will illustrate it by an example with which everyone is familiar and, once again, we will use rain as a source of random events. Consider a clothes line marked off into two segments, one being half the length of the other. When exposed to a shower, the longer one will, on the average, be hit by raindrops twice as often as the shorter. Of course, the absolute number of hits will depend upon the density of the rain, the length of the shower, and the thickness of the line. But the relative number of hits is actually a measure of segment length. If we found that one segment were hit twice as often as the other, we would judge it to be twice as long. The point separating the two segments is x hits from one end of the line and $2x$ hits away from the other end when the total number of hits on the line is recorded as $3x$.

With this analogy in mind, we may ask whether the distance between two genes determines the amount of recombination between them. If recombinations occur between the genes *arg* and *paba* 15 per cent of the time, and between genes *paba* and *thi* 30 per cent of the time, we would suppose the distance between *arg* and *paba* to be half the distance between *paba* and *thi*. But we would not know whether they were arranged in a linear order, as three points on a surface, or in a three-

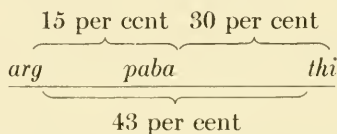
dimensional structure. If linear, we can immediately eliminate the possibility of the order



because the distance between *arg* and *thi* (43 per cent) is greater than that between *arg* and *paba*. If the order were



then the distance between *arg* and *thi* should be 15 per cent. But it was not; rather it was 43 per cent. The only spatial arrangement consistent with these data is:



because 43 is not significantly different from 45 per cent, the sum of 15 and 30 per cent. Arrangements other than linearity have been ruled out by the mapping of scores of genes in sequence in this way: the distances between them are always approximately additive. Thus genes are arranged in a linear order and the chance of recombination between them is fairly constant and a function of distance.

The method of mapping provides an image of the material basis of heredity as a linear structure like a rod along which the genes are located in order at specific loci. This image fits very well with the knowledge that the gene is a nucleic acid molecule which is also a linear structure, differentiated chemically along its length. It also fits the knowledge that the vast majority of known genes are located on chromosomes; these too are linear structures made, in part, of DNA.

GENES ON CHROMOSOMES

The chromosomal basis of Mendelian inheritance was established in the decade 1910–1920, primarily by Morgan and his students working on *Drosophila*, at Columbia University. Later a system for demonstrating more directly the connection between genetic and cytological behavior was found in the red bread mold *Neurospora*.

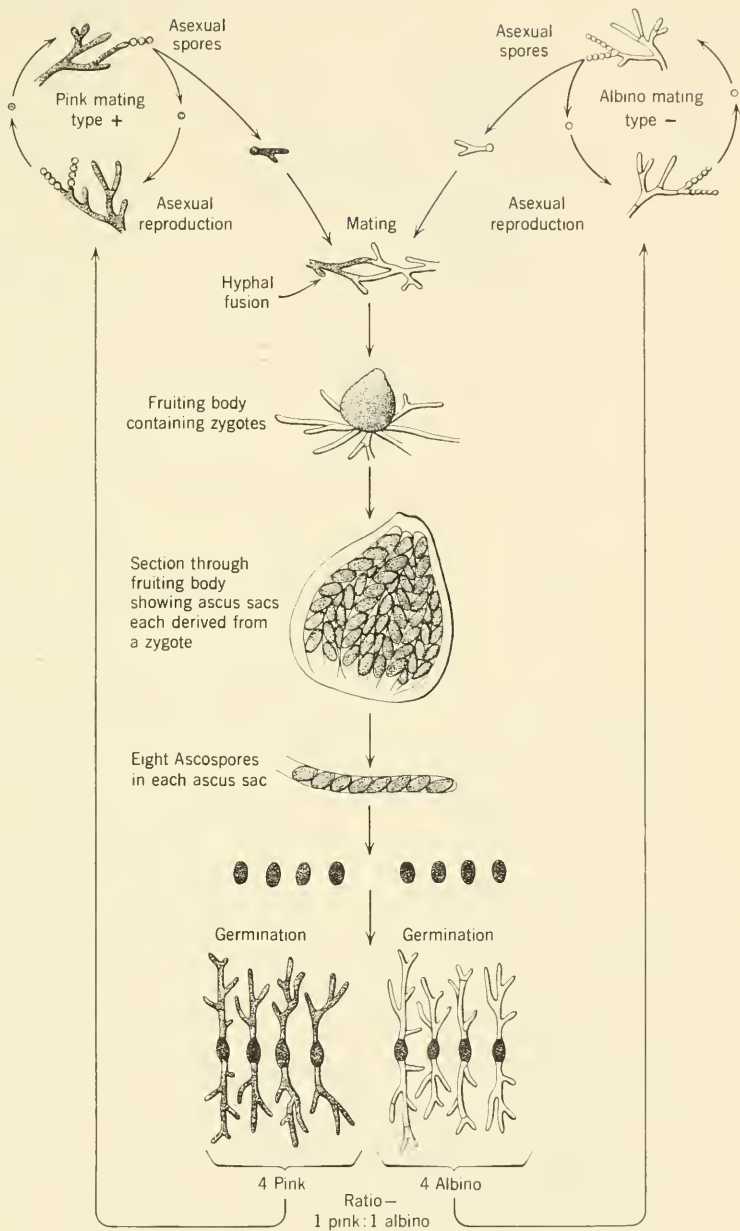
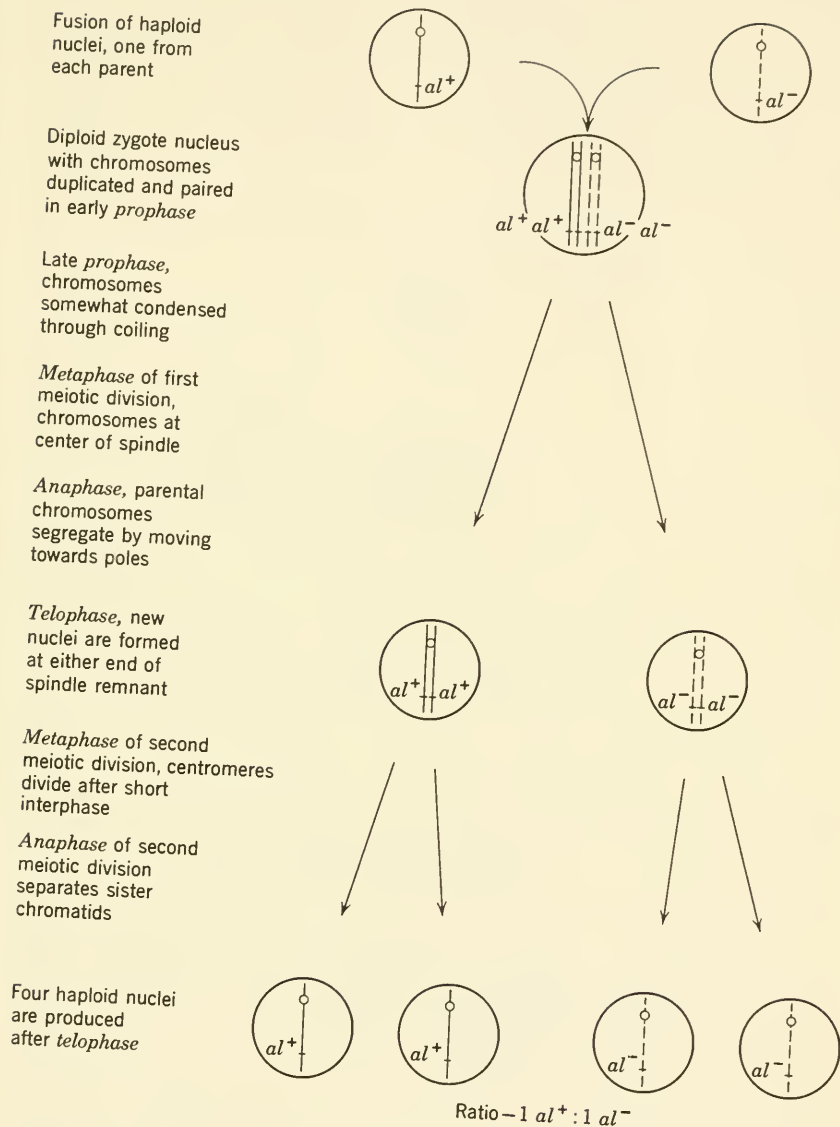


FIGURE 3.2. Diagram of the life cycle and the segregation of color in the red bread mold *Neurospora crassa*. It is a peculiarity of *Neurospora* that there are eight spores; the zygotes of other organisms usually produce four gametes each. The eight spores of *Neurospora* are almost invariably four pairs of identical sisters.



like, only one is represented for simplicity. In *Neurospora*, nuclear division is not followed by cell division.

This fungus consists of microscopic hyphal threads which, during asexual growth, branch, fuse, and spread to form a mycelial mat. Some hyphae form asexual spores which can germinate to give rise to a new mycelium. There are two mating types, mt^+ and mt^- (sometimes called A and a); when they fuse, so-called sexual spores are formed in a special organ called the fruiting body (Figure 3.2). These spores are the equivalent of the gametes of *Chlamydomonas* and, upon germination, they give rise to a mycelium. *Neurospora* is usually orange pink in color, but albino mutants occur. When the two are crossed, there is a 1:1 segregation of pink and white types among the sexual spores, similar to the segregation patterns of *Chlamydomonas*. How are these 1:1 ratios obtained?

The answer to this question lies in the behavior of the chromosomes. During the formation of gametes in most organisms the chromosomes segregate in two special divisions known as *meiosis*. Photographs of meiosis in the corn plant, *Zea mays*, are shown in Plates V, VI, and VII. Figure 3.3 shows a diagram of meiosis in *Neurospora*. The maternal and paternal nuclei each contain one set of chromosomes and are called *haploid*. When they fuse, the *zygote* nucleus so formed has both sets and is called *diploid*. The haploid number of chromosomes in *Neurospora* is seven; each is different in structure from the others. It is not known exactly when the chromosomes duplicate before meiosis in *Neurospora*, but during prophase in the diploid zygote nucleus each maternal chromosome pairs with its paternal homologue. The result is seven bivalents whose *centromeres* have not divided although the chromosomes have. (The centromere is that structure in the chromosome concerned with anaphase movement.) Thus each bivalent is composed of four strands or *chromatids* but only two centromeres, one for each parental homologue. The bivalents arrange themselves on the spindle at metaphase as tightly coiled structures; the centromeres, each with their two associated chromatids, pass to opposite poles at anaphase. In this way the homologous pairs of chromosomes are segregated. In the succeeding interphase the chromosomes do not duplicate again, but in the second meiotic division the centromeres divide, and the two chromatids which were already doubled in the prophase of the first meiotic division are separated. The result is four daughter nuclei, each haploid. If the chromosomes carry genes, it is clear that the orderly process of meiosis would segregate them in a 1:1 ratio, according to Mendel's first law. In *Neurospora* the nuclei produced by meiosis are arranged in the ascus sac in the order of their formation. Therefore, the four genes for pink color are in the nuclei of the spores at one end, and their alleles for albinism are at the other end.

When the spores germinate, the nuclei they contain divide by an

asexual process called *mitosis*. This process is quite different from meiosis in that each daughter nucleus has the same number of chromosomes as did the parental cell. Figure 3.4 contains a diagram of mitosis involving two homologous chromosomes in a diploid nucleus. In mitosis, homologous chromosomes do not pair; they are duplicated individually, the centromeres divide, and each pair of chromatids is arranged independently on the metaphase plate. At anaphase, the chromatids sep-

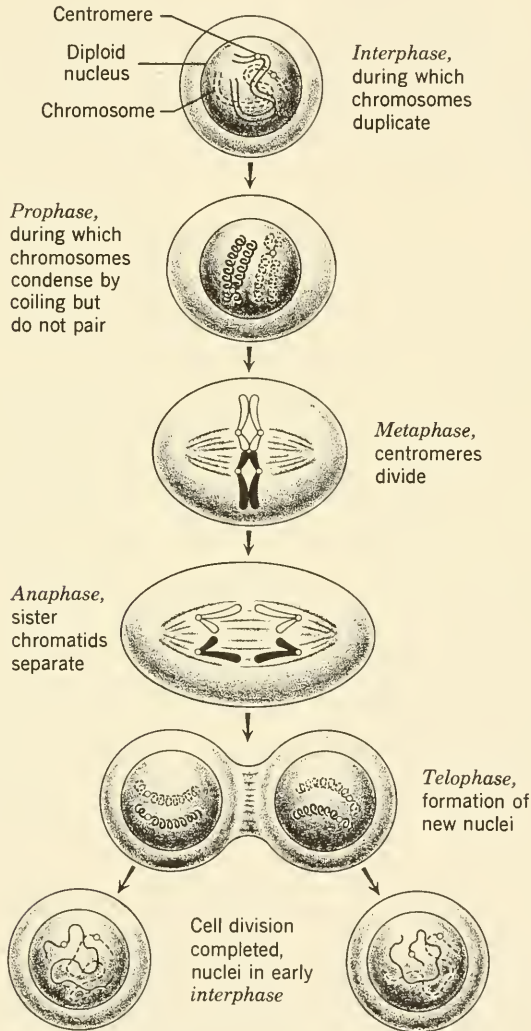


FIGURE 3.4. A diagram of mitosis in a diploid cell with one pair on homologous chromosomes.

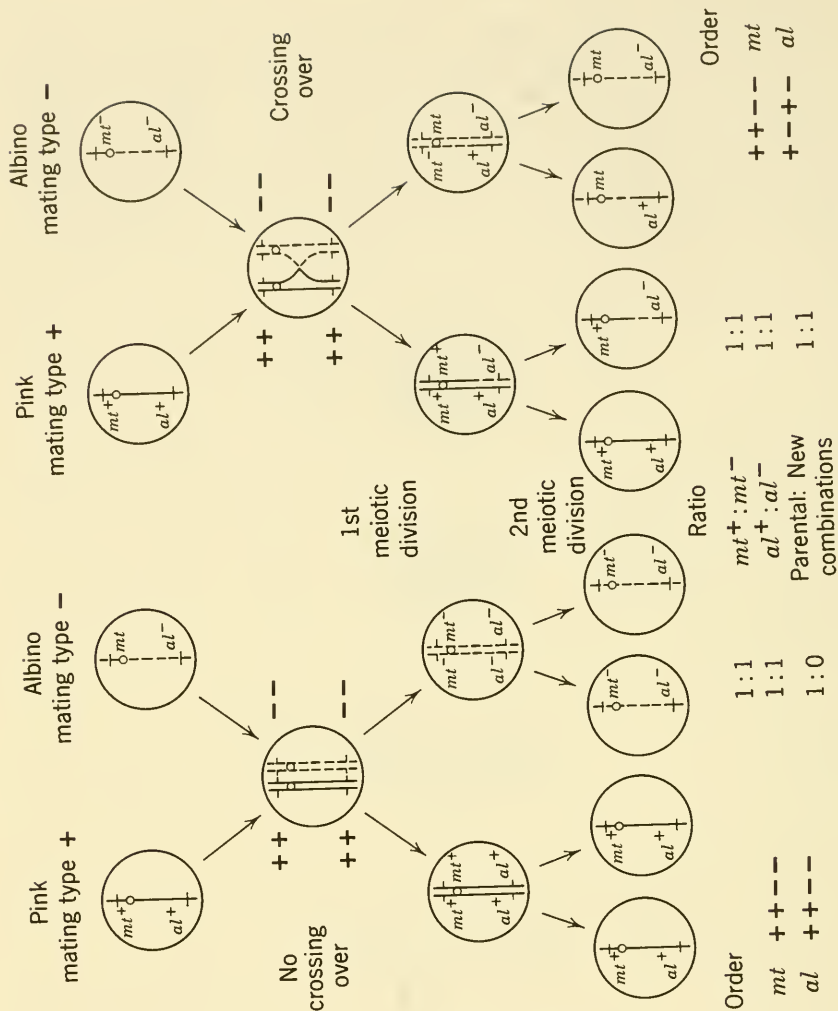


FIGURE 3.5. A diagram of linkage and crossing over during meiosis in *Neurospora* after a cross of a pink strain of + mating type with an albino strain of - mating type.

arate, following their centromeres to opposite poles. As a result, each of the two daughter nuclei that form after telophase has the same two chromosomes as did the original cell. Since every chromosome divides exactly, no matter how many make up each set, or how many sets are present, the daughter nuclei retain the same chromosomal complement as the cell from which they arose.

The important thing about mitosis is that it preserves the original chromosome number. Therefore, if some genes are located on chromosomes, mitosis will insure that during asexual vegetative reproduction each daughter cell receives the same genetic complement. Meiosis, on the contrary, is the mechanism for reducing the number of chromosomes in diploid cells by half and thereby segregating the members of homologous pairs into separate cells. The important distinction between mitosis and meiosis is that in mitosis there is one chromosome duplication for every division, whereas in meiosis there are two divisions with only one chromosome duplication.

Thus far we have considered only one pair of homologous chromosomes and the alleles they carry. But there are many genes on each chromosome, and many chromosomes. We will now consider the transmission of two pairs of alleles in a single cross. Since mitosis during asexual reproduction forms nuclei which have identical chromosome complements, their genetic constitutions are also the same. But at meiosis when the chromosome number is halved, parental combinations of chromosomes and genes are not always maintained. If the two allelic pairs of genes are on nonhomologous chromosomes, they are unlinked and will recombine among the gametes half the time, as shown in Figure 3.5. This is because nonhomologous chromosomes behave independently of one another at the first meiotic division. Thus, despite the fact that each pair of homologous chromosomes and the alleles carried segregate according to Mendel's first law in a 1:1 ratio, parental combinations and recombinations are produced in equal proportion. In this way the independent behavior of nonhomologous chromosomes accounts for the random assortment of the genes they carry, and, therefore, for Mendel's second law.

CROSSING OVER AND CHROMOSOME MAPPING

When two pairs of alleles show less than 50 per cent recombination, they are said to be linked. If the physical basis of linkage were the chromosome, one might expect either complete linkage or none at all. What is commonly found, however, is partial linkage or restricted recom-

ination. The unexpected phenomenon of genetic recombination between linked allelic pairs can be accounted for as shown in Figure 3.5. In the prophase of the first meiotic division, two of the four chromatids may exchange parts. As a result, one exchange forms two recombination chromatids. Each of these, together with the parental chromatid, still joined to the same centromere, passes to an opposite pole of the spindle. Thus two recombinant and two parental chromatids are assorted among the four gametes. This process of exchange is called *crossing over*. In the remainder of this chapter, we shall proceed on the assumption that recombination between linked markers results from chromosome exchange or crossing over, as shown in Figure 3.5. This model provides a basis for predicting the result of crosses, and can be tested experimentally. We will see that, in general, this model does satisfy the data for recombination between different genes. More rigorous proofs of the cytological basis of crossing over will be presented in Chapter 4. The molecular basis of recombination will be discussed in Chapter 7.

A single crossover event, occurring between the chromosomal loci of two allelic pairs of genes, recombines them. It is characteristic of crossing over that only two of the meiotic products are recombinant and that these are reciprocal in nature, e.g., $mt^+ alb^-$ and $mt^- alb^+$. Another consequence of crossing over is that the segregation of genes located distal to the point of its occurrence takes place at the second rather than at the first division. The order of genes within the ascus reflects this. Thus, when there is no crossing over between the locus alb and the centromere, half the time the order will be one of these two possibilities:

$$\begin{array}{c} alb^+ alb^+ alb^- alb^- \\ alb^- alb^- alb^+ alb^+ \end{array}$$

But when a crossover event occurs, there is an equal chance that the order will be any one of these four:

$$\begin{array}{c} alb^+ alb^- alb^+ alb^- \\ alb^- alb^+ alb^- alb^+ \\ alb^+ alb^- alb^- alb^+ \\ alb^- alb^+ alb^+ alb^- \end{array}$$

depending on how the chromatids segregate in the second division.

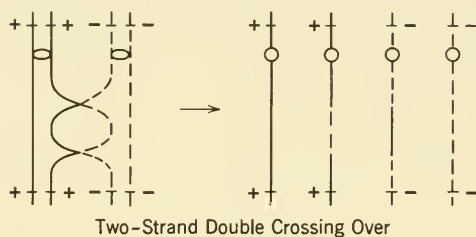
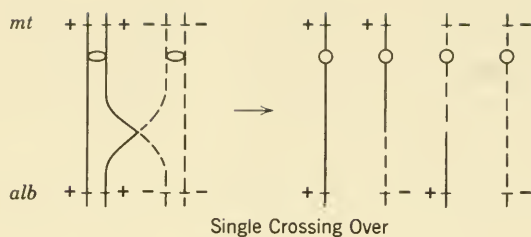
It is clear that second-division segregation changes the order only of genes that are distal to the point of crossing over when the centromere is used as a landmark. Thus, in the example in Figure 3.5, the mt gene shows the order $mt^+ mt^+ mt^- mt^-$ because no crossover event occurred between it and the centromere, but one did occur proximal to the locus

of *alb*, and the order $alb^+ alb^- alb^+ alb^-$ was found. Whenever a second-division segregation is observed, we know that crossing over was responsible for it. By counting the number of asci with second-division segregations, we can approximate the frequency with which crossover events take place between a gene and its centromere.

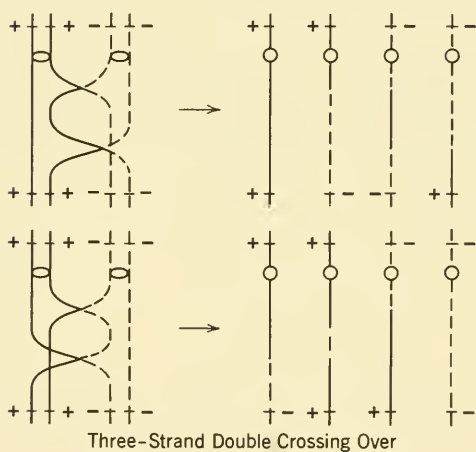
It is observed that some meioses do not involve a crossover event in a particular region under investigation whereas others do. For example, in an actual experiment with *Neurospora*, 54 asci were analyzed from a cross of $alb^- mt^+ \times alb^+ mt^-$. Of these 24 (or 44 per cent) showed first-division segregation of both allelic pairs and only parental combinations of them. We may suppose that no crossover event between either locus and the centromere happened in these meioses. Therefore, 56 per cent had such crossovers. Among these, if crossing over occurred between one or the other locus and the centromere, we would expect half of the spores produced to contain parental combinations of genes, and half to contain recombinations. The over-all frequency of new combinations expected can be calculated as follows:

	Frequency of Parental Combinations per Meiosis	Frequency of New Combinations per Meiosis	Frequency of New Combinations from All Meioses
0.44 Meioses with no crossover event	1.0	0	0
0.56 Meioses with crossover events	0.5	0.5	<u>0.28</u>
Total			0.28

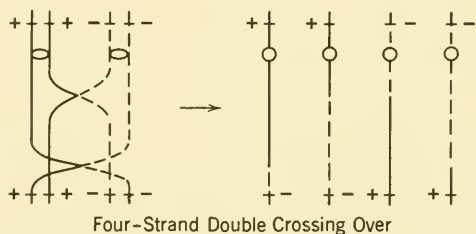
Actually, 0.31 new combinations were found among the 432 spores produced. The reason for the discrepancy between 0.28 and 0.31 is that three asci in which recombinants were found did not have the usual content of half parental and half new combinations; they had all new combinations in this arrangement: $alb^+ mt^+$, $alb^+ mt^+$, $alb^- mt^-$, $alb^- mt^-$. These asci may be explained if we consider the possibility of more than one crossover event between the centromere and a given locus. Figure 3.6 shows that four-strand double crossing over between either locus and its centromere will give the above result, i.e., all new-combination spores and only first-division segregation. We can see that, if the different kinds of double-crossover events occur at random in the proportion 1:2:1 as illustrated, the over-all frequency of new combinations is still 0.50 as it was with a single-crossover event. This is true for all numbers of crossover events, as is shown in Table 3.2. No matter what the



No new combinations
and first-division
segregation of color



50 Per cent new
combinations and
second-division
segregation of color



100 Per cent new
combinations and
first-division
segregation of color

FIGURE 3.6. The consequences of single and various kinds of double crossing over.

number of crossover events, the frequency of recombinants produced is 0.5, provided that the exchanges occur between the different chromatids at random.

The deviation from the expectation of 0.28 new combinations mentioned above is due in part to the fact that the three- and four-strand double-crossover events were recognized because they gave rise to recombinant spores, while the two-strand doubles which give rise only to parental combinations and to first-division segregations were not identified. The meioses in which they occurred would therefore be recorded as noncrossover. If the two-strand doubles equal the four-strand doubles, as the hypothesis of randomness predicts, and there were 3 of the latter among the 54 asci analyzed, then we may assume that there were 3 of the former. Thus 3 of the 24 asci showing first-division segregation and only parental combinations of genes were in reality caused by two-strand double-crossover events. To get the real number of asci in which no crossover events occurred, we subtract 3 from 24 and obtain 21. Subtracting 21 from 54 yields the number of asci in which at least one crossover event occurred. The frequency of meioses with crossing over is thus raised to 0.61 and the frequency of new combinations expected from all meioses to 0.31, which happens to be exactly what was found. In one of the 54 asci there were 0.5 new combinations but second-division segregation of both allelic pairs. This is expected from three-strand doubles if one crossover event occurs on each side of the centromere.

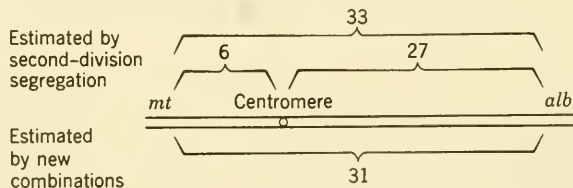
We can see in this sample of data from *Neurospora* an extraordinary correspondence between the behavior of genes and chromosomes. In

TABLE 3.2
The Relation between the Number of Crossover Events and the Frequency with which Different Types of Chromatids are Produced
(After Belling, 1931, *U. Cal. Publ. Bot.*, 16:311)

Number of Cross- over Events	Types of Chromatids					
	0 Non- Crossover (Parental Combina- tion)	1 Single Crossover (New Com- bination)	2 Double Crossover (Parental Combina- tion)	3 Triple Crossover (New Com- bination)	4 Quadruple Crossover (Parental Combina- tion)	5 Quintuple Crossover (New Com- bination)
1	0.5	0.5				
2	0.25	0.5	0.25			
3	0.125	0.375	0.375	0.125		
4	0.0625	0.250	0.375	0.250	0.0625	
5	0.03125	0.15625	0.3125	0.3125	0.15625	0.03125

Chapter 4 some cytogenetic tests will be described which demonstrate this correspondence in even more detail. Cytology and genetics have become a powerful team in the study of heredity and have made possible a much more direct and rapid analysis than formal genetics alone allows. The more recent recognition of nucleic acids as genetic material has permitted the further combination of chemical with genetic techniques. In all this the process of genetic exchange has been a most powerful tool; it has even been used for the analysis of the molecular fine structure of DNA, a feat as yet impossible by the use of chemical techniques alone (see Chapter 6).

One reason why recombination analysis has been so successful is that it allows the location of hereditary factors on genetic maps. When the factors are chromosomal genes, it is the chromosome that is mapped. In those cases where the products of a single meiosis can be collected for study, even the centromere may be given its position. In *Neurospora*, the frequency of second-division segregation measures the distance between the gene observed and the centromere because such segregations result from crossing over between them. To convert these frequencies into map units, they must be divided in half because each crossing over, although it results in one second-division segregation, yields only 50 per cent recombination. In actual experiments, 12 per cent of 3725 asci showed second-division segregation of the *mt* gene, and 54 per cent of 1263 asci showed second-division segregation of the *alb* gene. The *mt* locus is then 6 units, and the *alb* locus 27 units, from the centromere. But from this alone we cannot decide whether *mt* and *alb* are on the same or opposite arms of the chromosome. If the former were true, the distance between *mt* and *alb* would be 21; if the latter were true, the distance would be 33. This distance has already been estimated by the frequency of recombinations between *alb* and *mt*, which was 31 per cent. The close correspondence between 31 and 33 indicates that the chromosome map should read:



LIMITATIONS IN MAPPING

If, as genetic map construction assumes as a first approximation, crossing over occurs with a constant chance per unit of chromosome

length, then the crossover events would be distributed according to the Poisson equation (p. 48). The same parts of similar chromosomes would not cross over an identical number of times, but rather some would not cross over, some would cross over once, twice, and so on. For example, suppose that an average of one crossover event occurs per meiosis between two genes that are actually 50 units apart. If the crossover events are distributed at random according to the Poisson formulation and their average number, m , is one, then in about 37 per cent of meioses no crossing over will occur; in 37 per cent, one will occur; and in 26 per cent, two or more will take place. Now only crossover events give new combinations, so 63 per cent of the exchanges will produce recombinant chromatids. But both single and multiple crossover events yield only 50 per cent recombinant chromatids; 63 per cent $\times$ 50 per cent is 31.5 per cent. Thus, observation of the frequency of chromatids carrying new combinations of genes so far apart as 50 units would lead to a map distance of only 31.5. This error can be anticipated and the observed distance corrected to approximate the true one by the use of the equation:

$$y = \frac{1}{2}(1 - e^{-2d})$$

which relates the frequency of recombinant chromatids y to map distance d .

There is another way to avoid the error in mapping introduced by multiple crossing over. Because all single and multiple crossover events yield an average of 50 per cent recombination chromatids, genes very far apart on the same chromosome can recombine with a maximum frequency of 50 per cent. They behave in this way as if they were not linked. The smaller the distance between two genes, the less likely is a crossover event to occur; the smaller this chance, the smaller, relatively, is the chance of a multiple exchange. By mapping small distances, preferably in three-point crosses, and by adding many of them together, the large errors made in locating two distant genes can be avoided. Thus distances exceeding 50 map units can be measured.

But these methods involve assumptions about the constancy of the chance of crossing over along the length of the chromosome and the randomness with which different strands exchange with one another. It is known that deviations from these random expectations take place; they are called *interference* and are the subject of current investigation. If an exchange in one position along the length of a bivalent decreases the chance of a second exchange, then there is positive chiasma interference. Positive interference would occur if the chromatids were rigid so that one exchange inhibited other exchanges in close proximity, allowing them to take place only some distance away. But as we will see later,

the mechanism of exchange is not so simple and there may even be negative interference; this means that the occurrence of one exchange may make it *more* likely that another will occur in its proximity.

When evidence of interference is observed, the linear ordering of genes by the additive method may fail. In this case, other topological methods may be employed. For instance, in a three-factor cross, the middle gene can be separated from its two outside neighbors by a double exchange, one occurring between it and each of the terminal genes. Hence, the middle gene can be identified as that which occurs among recombinants least often in association with the other two from the opposite parent.

Ordered tetrads, such as the meiotic products of *Neurospora* and some other fungi and yeast, yield considerably more information than can be gained from the use of gametes that have been collected at random. Not only can independence in segregation or the degree of linkage between genes be derived from their analysis, but the 1:1 segregation of alleles is repeatedly verified (or exceptions are found) and the centromere is located and used as an additional marker. In three-point crosses not only the occurrence of double crossing over but also the specific strands involved in multiple exchanges may be observed directly. Unordered tetrads are more common among the algae, yeast, and fungi; these too have advantages, and special methods have been developed to exploit them. In most organisms, however, the meiotic products are not found in tetrads, so mapping is based upon the sampling of gametes at random.

Some genes cannot be located on chromosome maps by the techniques described; in fact, they do not show Mendelian segregation in crosses. A case in point is a gene for resistance to a high level of streptomycin in *Chlamydomonas*. It is not known to be linked to any other genes and it passes from one parent through the zygote into all four gametes. This and other cases that seem to involve nonchromosomal genes will be discussed in Chapter 9.

The genes to be discussed in Chapters 4–8 are located on chromosomes. Step by step their distribution along the whole length of a chromosome can be approximated. Such genetic maps have been checked cytologically by comparing the position of genes with the location of chromosome breaks that lead to chromosome aberrations. This can be done, for example, in the corn plant (Plate Vb) whose chromosomes show a pattern of enlargements (*chromomeres*), but much more easily in *Drosophila* (Plate VIIIa), where the giant salivary chromosomes appear cross-banded. The linear pattern of these bands can be compared with the pattern of gene loci on the genetic map as shown in Figure 3.7. The orders of genes are identical. The spacing does not exactly correspond, either because of some nonrandomness of crossing

over or because of structural variation along the chromosome, or both. The same is true of comparisons with usual somatic chromosomes where the position of X-ray-induced breaks cannot be ascertained so precisely.

A real difficulty with genetic mapping comes from the fact that the nature of the map depends upon the methods of analysis. In the first place, only mutations with detectable effects can be mapped. Secondly, only mutants which occur with a detectable frequency can be located. In haploid organisms, mutations with a lethal effect must be represented as gaps in the genetic map. Which of these classes a given mutant will occupy depends upon conditions. Take for example, the case of the *r* mutants of the bacteriophage *T4*, multiplying on a film of the bacterium, *Escherichia coli* strain *B*, on the surface of agar; the *r* phages form large plaques with a sharp margin; wild-type *r*⁺ phages

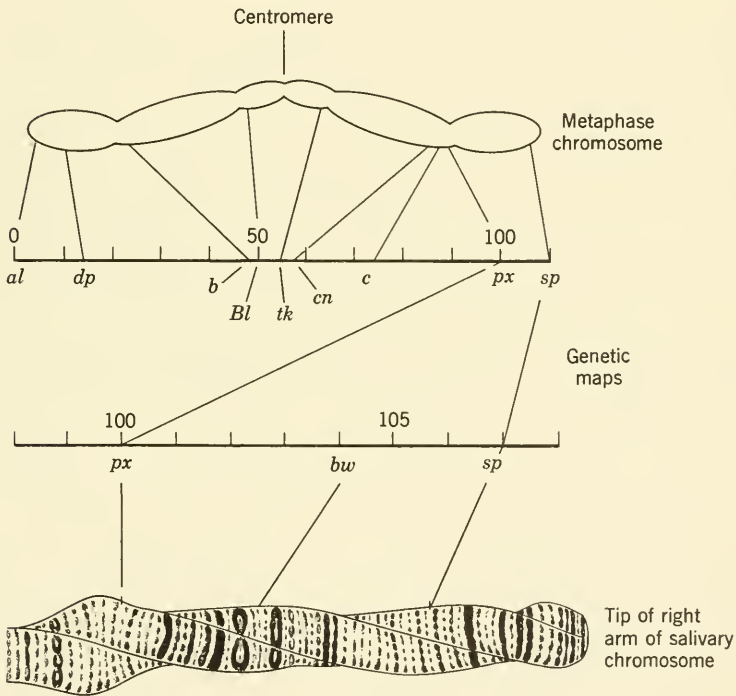


FIGURE 3.7. A comparison of genetic with cytological maps of the second chromosome of *Drosophila melanogaster* (from Dobzhansky, in Duggar et al., 1936, *Biological Effects of Radiation*, Vol. 2, p. 1167, New York, McGraw-Hill; and Bridges, 1937, *Cytologia, Fujii Jub.*, 2:745).

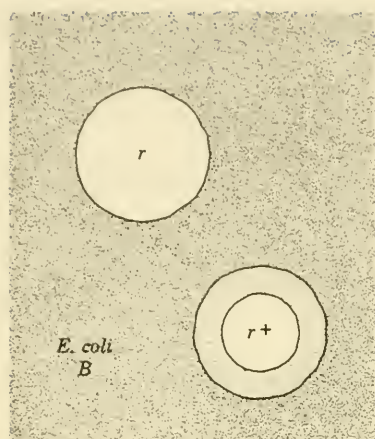


FIGURE 3.8. The appearance of plaques of r and r^+ bacteriophage multiplying on a film of the bacterium, *E. coli*, strain *B*. A plaque is the clear area formed by the lysis (disintegration) of the bacterial host.

form small plaques with a fuzzy, slightly turbid margin (Figure 3.8). Types of r mutants exist which are differentiated by their behavior on strains other than *B* (Table 3.3). Mutants of class rI form large clear plaques on all three tester strains; those of class rII are mutant on strain *B*, wild type on strain *S*, and form no plaques on strain *K*; mutants of class $rIII$ form wild-type plaques on strains *S* and *K*. At the bottom of Table 3.3 are shown the maps that would be found if studies were restricted by the use of only one tester strain of bacterium. On strain *B* all three mutants can be recognized as such and would be located on the map; on strain *S* only the rI mutants could be located; on strain *K*, rI mutants would be mapped as such but since a few rII mutants can multiply on *K* to form minute plaques, these would be located at a site probably called the minute locus.

Beyond all this, the arbitrary choice of the wild type determines the appearance of the map. If there were only one allelic state that gave the rII^+ character and many rII alleles that could arise from it by mutation, then there would be one rII^+ locus on the genetic map if r^+ were chosen as wild type. If, on the other hand, a particular rII allele were chosen as wild type, then each new rII mutant found which could be separated from the others by recombination would be given a separate, albeit neighboring, locus. We will see in Chapter 6 that alleles can be fragmented by recombination so this is a real possibility. But despite the relativity of genetic maps, their construction in great detail is in itself one of the most powerful methods for the analysis of the behavior and fine structure of the chromosome and of the gene.

TRANSMISSION GENETICS OF DIPLOIDS

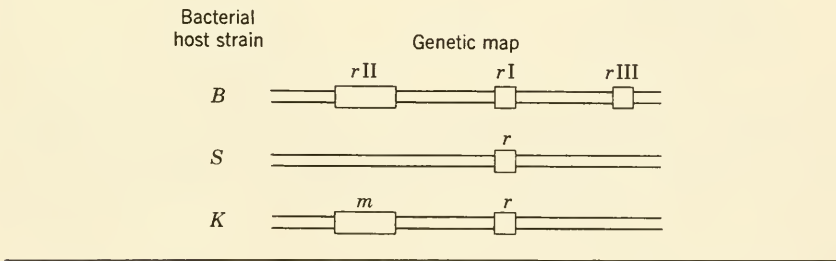
Mendel's laws hold for all organisms but their consequences appear differently according to whether the gametes may be analyzed directly. Figure 3.9 contrasts the life cycles of some haploid and diploid organisms. The baker's yeast, *Saccharomyces cerevisiae*, is intermediate; part of its life cycle is in a diploid and part in a haploid phase because both the gametes and the zygotes multiply asexually. Hence in either phase enough cells may be collected to observe phenotypic traits and to infer the genotype. In haploid forms such as *Chlamydomonas* and *Neurospora* only the gametes provide enough cells by multiplication to make such analyses convenient. In multicellular forms it is the zygote which divides asexually, the associated mitoses insuring a population of diploid somatic cells. The gametes themselves do not usually divide.

Nonetheless, meiosis in diploid organisms takes place essentially as in haploids (Figure 3.3). As a result the alternative members of a pair of alleles are distributed among the sperm or eggs in a 1:1 ratio. Usually the genotypes of the gametes cannot be determined directly, but crosses must be employed and deductions made from the phenotypes of the in-

TABLE 3.3
Plaque Morphology of Mutants of Bacteriophage T4 on Various Host Bacteria and Dependence of Genetic Map on Choice of Host

(After Benzer, in McElroy and Glass, 1957, *The Chemical Basis of Heredity*. Baltimore: The Johns Hopkins Press, p. 70)

Phage Strain	<i>E. coli</i> Host Strain		
	<i>B</i>	<i>S</i>	<i>K</i>
Wild	wild	wild	wild
<i>r</i> I	<i>r</i>	<i>r</i>	<i>r</i>
<i>r</i> II	<i>r</i>	wild	<i>m</i>
<i>r</i> III	<i>r</i>	wild	wild



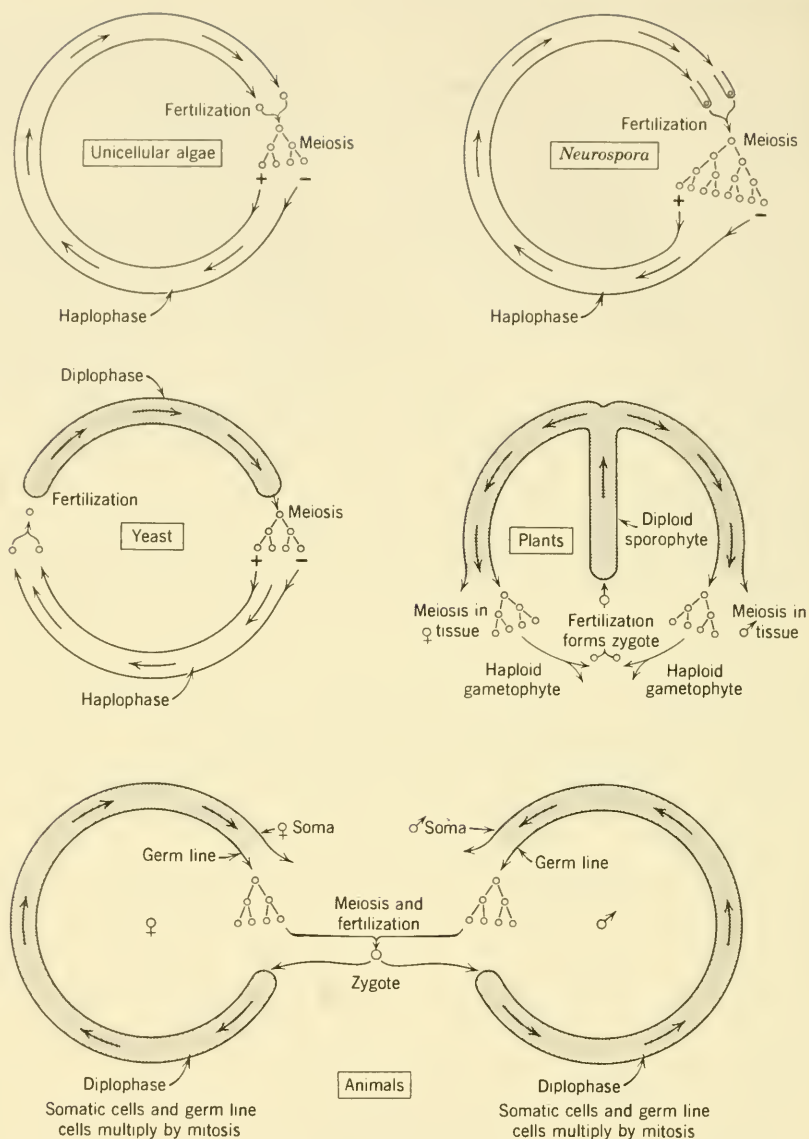


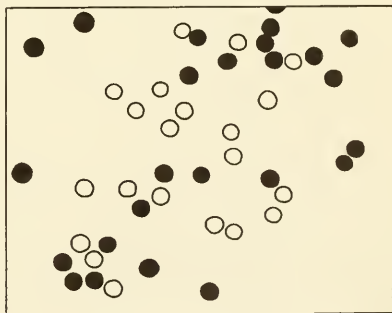
FIGURE 3.9. Generalized diagrams of the life cycles of haploid and diploid organisms illustrating different systems of sexuality and mating. The symbol ♀ stands for female and represents the mirror of Venus; the symbol ♂ is for male and represents the spear of Mars.

dividuals produced. In the corn plant, however, the segregation of one pair of alleles can be observed directly in the gametes. Members of the waxy-starchy gene pair have different effects on the phenotype of pollen; pollen grains carrying the starchy gene form starch and stain blue with iodine, whereas those that possess the waxy gene contain a red staining starch. Pollen produced after the segregation of this pair of alleles shows statistically a 1 : 1 ratio of the two phenotypes (Figure 3.10). Among micro-organisms, tetrads including all four products of meiosis are analyzable so that Mendel's first law can be directly verified.

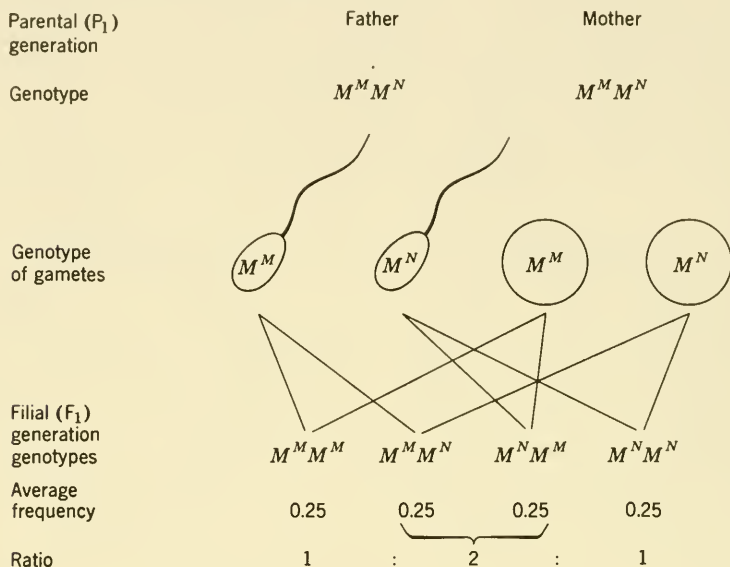
In mating diploid organisms, sperm, pollen, and eggs are necessarily sampled at random. Any two sperm are not likely to be members of the same quartet of meiotic products because of the few progeny studied relative to the tremendous number of sperm produced. In the case of eggs, no two come from the same quartet because only one product of each meiosis survives to form an egg. The union of sperm and eggs is random and does not, in general, depend upon the genotype of the gamete. To illustrate some ratios of diploid genotypes, we will use data on the inheritance of the M and N blood groups in man. Individuals of M blood group produce gametes that are genetically homogeneous and carry a gene for that character. We refer to the gene as M^M ; fusion of an M^M sperm with an M^M egg results in an M^M/M^M individual which can, in turn, produce only M^M sperm or eggs. The same is true for individuals of N blood group, whose genotype is written M^N/M^N . These conclusions are drawn from the results of the matings which are shown in Table 3.4. The mating $M \times N$, involving fusion of gametes of M^M composition with those of M^N composition, should then give only MN individuals, which in fact, it does.

Diploid organisms possessing identical alleles at a given chromosomal location (*locus*) are called *homozygotes* (e.g., M^M/M^M) whereas those having different alleles at a locus are called *heterozygotes* (e.g., M^M/M^N).

FIGURE 3.10. The segregation of the starchy and waxy alleles among pollen produced by a maize plant heterozygous for this gene pair (after Demerec, in: Sinnott, Dunn, and Dobzhansky, 1958, *Principles of Genetics*, p. 56, New York, McGraw-Hill).



At meiosis a heterozygote produces gametes which have either one allele or the other. The sperm and eggs of an M^M/M^N heterozygote are either M^M or M^N . If these two types are produced in a 1:1 ratio, and if sperm combine with eggs at random, then there are in equal frequencies four possible diploid combinations at fertilization:



The two heterozygotes are indistinguishable; therefore the proportions among the progeny of an $MN \times MN$ mating should be on the average 1:2:1. Table 3.4 shows that this is indeed a fact. A confirmation of the genetic heterogeneity of the gametes of heterozygotes is found in the results of a cross of a heterozygote with a homozygote; there should be only two diploid combinations in equal proportions and this is found to be the case.

The heterozygotes in the example just given expressed both alleles and formed M and N red blood cell antigens. More often one allele dominates. For example, an individual heterozygous for genes determining brown and blue eye color has brown eyes; the gene for brown eyes is *dominant* and that for blue eyes is *recessive*. The heterozygote cannot readily be distinguished from a brown-eyed homozygote, showing that individuals may have indistinguishable phenotypes although their genotypes differ. This circumstance determines the ratio of phenotypes observed among the progeny. Consider a mating of a homozygous brown-eyed individual (B/B) with a homozygous blue eyed one (b/b). Their F_1 offspring would all be brown-eyed but heterozygous (B/b). If

these heterozygotes were to mate at random with individuals of identical genotypes, the result would be the same as if they had inbred among themselves. There would be, on the average, 75 per cent brown-eyed progeny (B/B , B/b , and b/B); the remaining 25 per cent would be blue-eyed individuals (b/b) in whom the recessive b genes, hidden in the F_1 heterozygotes, were able to express themselves because of the absence of the dominant B gene.

When the F_1 heterozygotes mate with individuals of B/B genotype, it would be as if they had backcrossed with the B/B parent; only brown-eyed (B/B and B/b) offspring would result. Backcrosses of $B/b \times b/b$ can give only B/b and b/b offspring in equal proportions. The 3:1 ratio of phenotypes in the F_2 generation and the results of the backcrosses are a demonstration of Mendel's first law because they are a necessary consequence of the 1:1 segregation of genes.

Matings between *truly* blue-eyed individuals can give rise only to blue-eyed children. The qualification refers to the fact that eye colors are sometimes misclassified. This is because there are more eye colors than blue and brown, and other genes act as modifiers. The incautious inference of genotype from phenotype must not be the cause of accusations of inconstancy of affection.

Mendel's second law of random assortment also predicts specific ratios of genotypes and phenotypes in diploids. Individuals heterozygous for two unlinked genes produce four different kinds of sperm or eggs in equal proportions. From these, sixteen (4^2) different combinations of

TABLE 3.4
Inheritance of M and N Blood Groups in Man

(From Wiener and Wexler, 1958, *Heredity of the Blood Groups*.
Grune and Stratton, New York, p. 43)

Blood Groups of Parents	Number of Families	Blood Groups of Children		
		M	MN	N
$M \times M$	153	326	1°	0
$N \times N$	57	0	0	106
$M \times N$	179	1°	376	0
$MN \times MN$	377	199	405	196
$M \times MN$	463	499	473	1°
$N \times MN$	351	3°	411	382

°There are six evident exceptions among the 3379 cases shown in the table. They could have been mistakes in blood typing, instances of mutation, or cases of illegitimacy. Considerations of these possibilities may lead to an intelligent solution of questions of disputed parentage.

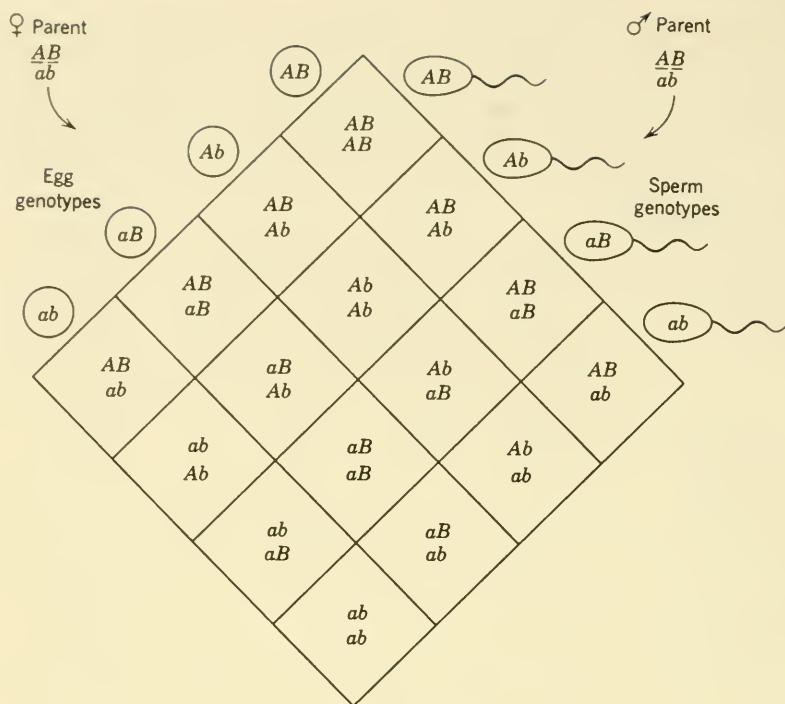


FIGURE 3.11. A chart showing the combinations of genes possible in the zygotes formed from a mating of individuals heterozygous for the same two nonallelic independently assorting genes.

two can be made. As an example, we will consider a mating between two individuals simultaneously heterozygous for genes determining the ability to utilize the sugar, galactose, and determining eye color. These individuals have the genotype, $A/a B/b$. The four kinds of meiotic products would have the genetic compositions AB , Ab , aB , and ab . The possible results of fertilizing eggs of these sorts at random with sperm of the same genotypes are shown in Figure 3.11. It will be seen that a ratio of 9:3:3:1 is expected for the phenotypes brown-eyed, galactose utilizers; brown-eyed, galactose nonutilizers; blue-eyed, galactose utilizers; blue-eyed, galactose nonutilizers.

The same result will be obtained by writing out the multiplications implicit in the expression $[(A+a)(B+b)]^2$. For example, $(A+a)(B+b) = AB + Ab + aB + ab$; multiplying the latter term by itself gives the genotypes in the table. This method is convenient when

larger numbers of independently segregating genes are involved in a cross.

Because of the problem of dominance, we are concerned with the phenotypes of the offspring as well as the genotypes. A short-cut method may be used. Considering one factor at a time, we know that the recessive phenotype will occur with a frequency of $\frac{1}{4}$. Then:

$$\begin{aligned}\frac{1}{4} \times \frac{1}{4} &= \frac{1}{16} \text{ blue-eyed, galactose nonutilizers} \\ \frac{1}{4} \times \frac{3}{4} &= \frac{3}{16} \text{ blue-eyed, galactose utilizers} \\ \frac{3}{4} \times \frac{1}{4} &= \frac{3}{16} \text{ brown-eyed, galactose nonutilizers} \\ \frac{3}{4} \times \frac{3}{4} &= \frac{9}{16} \text{ brown-eyed, galactose utilizers}\end{aligned}$$

Next let us see how linkage and crossing over affect the transmission of genes in diploid organisms. Suppose the genes for blue eyes and galactose utilization in man were linked and not, as was presumed in the case illustrated in Figure 3.11, on different chromosomes. Then, if crossing over occurred in 50 per cent of the meioses, the haploid gametes of individuals heterozygous for these genes (AB/ab) would not occur with the genotypes AB , ab , Ab , and aB in equal numbers. On the contrary, their frequencies would be 0.375, 0.375, 0.125, and 0.125 respectively ($0.5 \times 0.5 = 0.25$, which is the frequency of old and of new combinations produced in meioses in which crossing over occurs; the frequency of each type of new and old combination is, therefore, 0.125). The last two classes would be the new combinations. Matings of individuals heterozygous for these genes would give a complicated ratio of types among their offspring. Multiplying the expression $0.375AB + 0.375ab + 0.125Ab + 0.125aB$ by itself would give a ratio of brown-eyed utilizers: brown-eyed nonutilizers:blue-eyed utilizers:blue-eyed nonutilizers of about 64:11:11:14. This might be distinguished from the 9:3:3:1 ratio which is achieved when the genes are unlinked. But the recombinations can be more simply detected if the heterozygote is crossed to a recessive homozygote whose gametes are all ab in composition, a so-called test cross. In this case, 0.375 of the offspring would be brown-eyed utilizers (AB/ab), 0.375 would be blue-eyed nonutilizers (ab/ab), 0.125 would be blue-eyed utilizers (Ab/ab), and 0.125 would be brown-eyed nonutilizers (aB/ab), and the frequencies of recombinant gametes from the heterozygote could be determined directly.

Not many human families are large enough to realize even simple ratios which are only the averages found in ideal populations of infinite size. Man is an inconvenient object for genetic study, although we want more to know about his genetics than any other. He reproduces slowly and has small families so that the offspring of many parents of identical genotype must be counted to get a large enough set of data in which to

observe a segregation ratio. But even this is difficult to do because human matings, fortunately, cannot be made at the will of an experimenter. As Beadle has written, the choice of a man's mate is not often based on a desire to contribute to the knowledge of heredity and, furthermore, it is inconvenient to subject him to a complete chemical analysis. As a consequence, human heredity must be studied by the examination of family trees and by other indirect methods. With "lower" plants and animals, however, sufficiently large populations of offspring can be collected from identical matings, and ratios such as the type 9:3:3:1 can be tested directly. Three-factor crosses yield, of course, more complicated ratios. These ratios can be worked out mechanically by the same methods we have employed thus far. They will be described in any textbook of classical genetics. The more complex the ratios become, the larger the number of siblings that must be collected to establish them.

It should be evident, however, that complex as the combinations resulting from the assortment of many genes may be, the behavior of the individual genes is simple and is only a consequence of the chromosome mechanics of meiosis and fertilization. We shall next consider some of the more specific correlations between genes and chromosomes.

BIBLIOGRAPHY

- Barratt, R. W., D. Newmeyer, D. D. Perkins, and L. Garnjobst, 1954. Map construction in *Neurospora crassa*. *Adv. in Genetics*, **6**:1-93.
- Bridges, C. B., and K. S. Brehme, 1944. The mutants of *Drosophila melanogaster*. *Carnegie Inst. Wash. Publ.* 552, 257 pp.
- Catcheside, D. G., 1951. *The Genetics of Microorganisms*. New York, Pitman, 223 pp.
- McLeish, J., and B. Snoad, 1959. *Looking at Chromosomes*. London, Macmillan, 88 pp.
- Perkins, D. D., 1955. Tetrads and crossing over. In: *Symposium on Genetic Recombination*, *J. Cell. Comp. Physiol.*, **45**, Suppl. 2:119-149.
- Sager, R., 1955. Inheritance in the green alga *Chlamydomonas reinhardtii*. *Genetics*, **40**:476-489.
- Srb, A. M., and R. D. Owen, 1952. *General Genetics*. San Francisco, Freeman, 561 pp.
- Stern, C., 1960. *Principles of Human Genetics*. (2nd ed.). San Francisco, Freeman, 753 pp.
- Swanson, C. P., 1957. *Cytology and Cytogenetics*. Englewood Cliffs, N. J., Prentice-Hall, 596 pp.

4 Cytogenetic Correlations and Crossing Over

Genetic mapping is made possible by the occurrence of recombination between linked genes. In succeeding chapters, we shall see how useful a procedure mapping can be in the investigation of genetic material. In this chapter, we are concerned with the process of recombination of linked genes (crossing over) as it occurs in sexual organisms and with some of the kinds of aberrant behavior of which chromosomes are capable. Most of our information comes from the correlated chromosomal and genetic analysis of breeding behavior in *Drosophila* and in the corn plant, *Zea mays* (maize). A group of brilliant investigations in the classical period of cytogenetic research, centered in the 1920's and early 1930's, established the ground rules of chromosome behavior as the basis of the segregation and recombination of Mendelian factors.

These investigations established many of the regularities of crossing over, but did not elucidate the mechanism of the process. It now seems likely that the mechanism will require analysis at the molecular level. Research in that direction will be discussed in Chapter 7. Here we shall consider what has been learned about crossing over from cytogenetic correlations.

Historically, this area of research began with the demonstration that linkage groups established by genetic mapping could be correlated with the cytologically observable chromosomes. Bridges first showed that a peculiar pattern of genetic segregation was the result of an aberrant type of chromosome segregation called nondisjunction. The regularities of sex-linked inheritance in *Drosophila* had been established previously by Morgan. As shown in Figure 4.1, when a white-eyed female is crossed with a red-eyed male, all F_1 female progeny are red-eyed and all males are white-eyed. In the F_2 produced by brother-sister matings, both

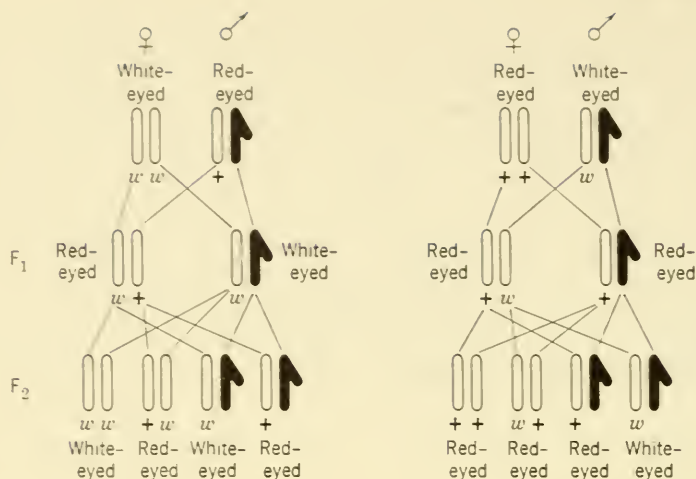


FIGURE 4.1. Sex-linked inheritance of eye color in *Drosophila*. Reciprocal crosses of white-eyed and red-eyed flies show the pattern of inheritance of a gene carried on the X chromosome. This type of inheritance results from the absence of w (and other genes which are similarly inherited) from the Y chromosome. The genotype is thus determined by the segregation of the X chromosome (after Sturtevant and Beadle, 1939, *And Introduction to Genetics*, Philadelphia, Saunders, pp. 30-31).

sexes segregate 1:1 for eye color, rather than 3:1. In the reciprocal cross, between a homozygous red-eyed female and a white-eyed male, all F_1 progeny are red-eyed, but in the F_2 all the females and half of the males are red-eyed. It should be recalled that in *Drosophila*, the female carries two X chromosomes, and the male an X and a Y; sex determination is a consequence of the segregation of X and Y into separate sperm in meiosis.

Bridges investigated a strain of white-eyed females whose progeny did not all follow this pattern. In crosses of these females with red-eyed males, about 4 per cent of the female progeny were white-eyed like their mothers and about 4 per cent of the males were red-eyed like their fathers, directly violating the regularities of sex-linked inheritance which had just been established.

In his doctoral dissertation, Bridges demonstrated that this unusual pattern of segregation resulted from the special chromosome composition of this white-eyed female strain. It had originated by abnormal egg formation in which the two X chromosomes stayed together in meiosis, resulting in the appearance of two unusual classes of eggs, XX and O. This process is called *primary nondisjunction*. On fertilization, four abnormal classes of progeny are produced:

From the White-Eyed Female Parent	From the Red-Eyed Male Parent	
	X	Y
XX	XXX lethal females	XXY <i>white-eyed females</i>
O	XO sterile males	YO lethal males

Bridges proved that the white-eyed female strain he had been studying, which gave about 8 per cent unexpected progeny, was actually XXY in chromosome constitution, and that in its meioses *secondary nondisjunction* occurred, giving rise to four kinds of eggs with the frequencies shown:

From the White-Eyed Female Parent	From the Red-Eyed Male Parent	
	X	Y
X (ca. 46%)	XX red-eyed female	XY white-eyed male
XY (ca. 46%)	XXY red-eyed female	YYY white-eyed male
XX (ca. 4%)	XXX lethal female	XXY <i>white-eyed female</i>
Y (ca. 4%)	XY <i>red-eyed male</i>	YY lethal

Thus, in this nondisjunctional strain, about 4 per cent of the viable females received both X chromosomes from their mother, and about 4 per cent of the viable males received their one X chromosome from their father.

This demonstration of nondisjunction of the X chromosome in *Drosophila* represents the first instance in which the aberrant segregation of a genetic factor was correlated with a chromosomal aberration, thereby rigorously establishing the chromosomal basis of gene segregation. In maize, the linkage groups were first correlated with particular chromosomes by McClintock with the use of trisomic ($2n+1$) stocks, i.e., plants in which one of the ten chromosomes is present three times instead of twice. It should be appreciated that these first cytogenetic correlations were not only difficult to establish experimentally but also represented an important conceptual advance, laying the foundation for the intricate and sophisticated analyses of chromosome rearrangements which followed.

CHROMOSOMES AS SEEN WITH THE LIGHT MICROSCOPE

Chromosomes were first described in the 1870's, and cytologists have been observing them ever since. There is by now a tremendous descriptive literature based upon studies with the light microscope. The detailed morphology of each chromosome is itself an aspect of cell heredity. Chromosomes have an individuality at the level of the light

microscope, in terms of length, position of the *centromere*, arrangement along the chromosome of small thickenings called *chromomeres*, and of larger ones called *knobs*, of various constrictions at particular locations, and of differential intensity of staining with basic dyes (which react with nucleic acids).

Very detailed observations of chromosome morphology are possible with the remarkable many-stranded chromosomes found in some insect larvae, the best known being the salivary gland chromosomes of *Drosophila* larvae (Figures 3.7 and Plate VIII). These chromosomes are about 100 times the length of metaphase chromosomes of adult *Drosophila* tissues, and contain about 1024 times as much DNA as do the corresponding chromosomes of adult diploid cells. The salivary gland chromosomes are polytene, each consisting of a parallel array of 1024 strands, with each strand corresponding to one chromosome of the adult cell. The homologous chromosomes are synapsed and show the same precision of point-for-point pairing found in meiotic pachytene. As seen in Figure 4.2, each salivary chromosome has a distinctive pattern of deeply staining bands separated by faintly staining regions. These patterns have provided the basis for detailed cytological maps which have been correlated with genetic maps derived from recombination data (Figure 3.7).

Another extraordinary type of chromosome is that found in the unfertilized eggs (oöcytes) of many amphibians and other vertebrates. These so-called lampbrush chromosomes attain lengths of over a millimeter during meiotic prophase. Electron microscope studies of their fine structure will be discussed in Chapter 7. No cytogenetic correlations have been made with this material because, in general, the species with good lampbrush chromosomes have not been well suited to genetic analysis.

Cytogenetic correlations in *Drosophila* have been established primarily with salivary gland chromosomes, and in other organisms, primarily with the chromosomes of meiotic stages. In maize, for example, as shown in Plate Vb, the morphological individuality of each of the ten chromosomes is readily distinguishable in the prophase stages of meiosis.

Associated with the chromosomes is a body (sometimes more than one) called the *nucleolus*, which consists mainly of protein and RNA, and which breaks down in every mitotic cycle and reappears at telophase. The reorganization of the nucleolus has been shown to be under the control of a special chromosomal region, called the nucleolar organizer. McClintock studied a maize strain in which this region was split by X-rays and one part was translocated to another chromosome. She found that each part independently organized some nucleolar material.

Chromosomes contain regions which stain intensely with basic dyes and have been called *heterochromatic*, in contrast with faintly staining areas, referred to as *euchromatic*. A popular interpretation of this staining difference has been in terms of a differential condensation or coiling of the chromosome backbone. The fact that chromosomes do coil is evident at the level of the light microscope, as seen, for example, in *Tradescantia*. In the *Drosophila* salivary chromosomes, heterochromatic regions are seen near the centromeres of chromosomes II, III, and X, which are coalesced forming the *chromocenter*; and the entire Y chromosome is considered heterochromatic. Bits of heterochromatic material are scattered throughout the *Drosophila* genome as well as concentrated in the chromocenter. The same is probably true in most organisms.

Two features have been noted in the behavior of heterochromatic regions: (1) Genes are rarely found there, and (2) the expression of dominant genes from euchromatin is often blocked when such genes are moved into heterochromatic regions by chromosome rearrangements after X-irradiation in *Drosophila*. This phenomenon, called position effect, was the first evidence of gene interaction at the chromosome level, an exceedingly important problem to be discussed in Chapter 11. In many organisms B chromosomes which are heterochromatic are present in some strains and not in others. They are distinguished by the fact that no change in phenotype is evident when their number is increased or diminished by appropriate crosses. In many species, the sex chromosomes are heterochromatic. In *Drosophila* very few genes have been located on the Y chromosome, yet it plays a decisive role in the formation of normal sperm.

There are no generally accepted criteria for the identification of heterochromatin, and it is possible that a number of differently functioning regions have been grouped under this heading. Those that exert position effect might constitute one group, but this criterion would be useful only in organisms such as *Drosophila* in which position effect can be readily studied. Recent evidence that heterochromatic regions undergo duplication at a different time in the mitotic cycle than do euchromatic regions might provide another criterion if preliminary observations are confirmed in other organisms. One may sum up the present situation as follows: neither the chemical, the structural, nor the functional properties of heterochromatin have been established, but nonetheless there is a widespread feeling among cytologists and geneticists that heterochromatic material plays some biologically distinct and significant role.

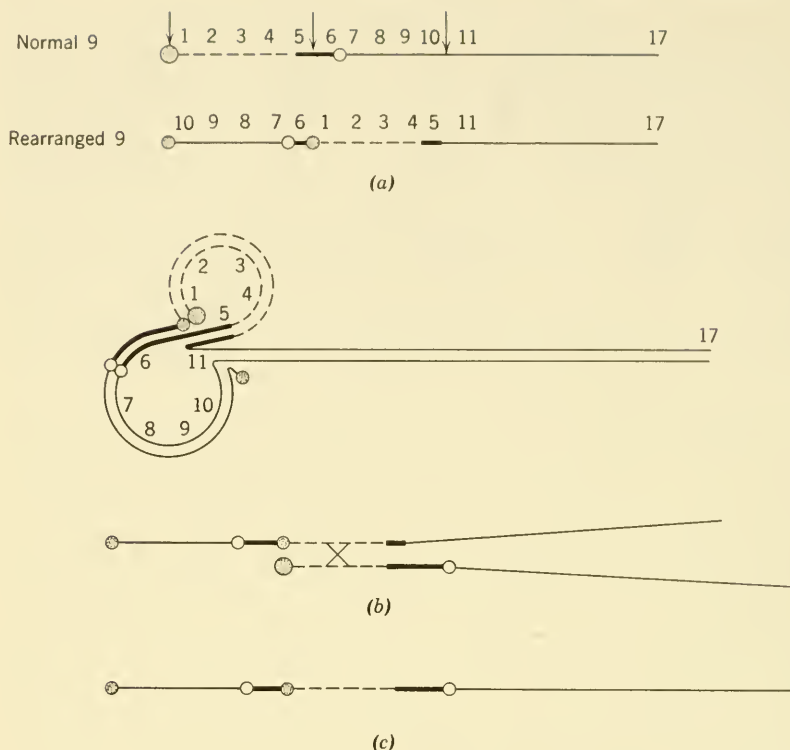


FIGURE 4.2. Origin of dicentric chromosome in maize (after B. McClintock, 1941, *Genetics*, 26:234).

(a). Comparison of chromosome 9 before and after rearrangement resulting from three X-ray-induced breaks denoted by arrows. Stippled circles represent knobs, clear circles represent centromeres, the dotted region 1-5 indicates position of crossover, and the heavy lines represent heterochromatic regions.

(b). Pachytene pairing of normal 9 with rearranged 9 showing point-to-point pairing configuration, and the position of crossover which gave rise to the dicentric chromosome shown in (c).

(c). Dicentric chromosome resulting from the crossover shown in (b).

CHROMOSOME BREAKAGE AND REARRANGEMENTS

Judging from the similarities in chromosome morphology and in genetic linkage from strain to strain of most species, one might infer that the chromosome is an exceedingly stable structure. It came as something of a surprise, therefore, when geneticists found that chromosomes were readily able to undergo breakage and rearrangement. De-

tailed investigations of these processes became possible after the demonstration that X-rays could break chromosomes. One of the most vivid demonstrations of chromosome fragility was provided by McClintock's analysis in maize of a repeating cycle of breakage-fusion-bridge formation in the outermost cell layer (aleurone) of the endosperm (that part of the corn kernel in which starch is stored).

Many of the studies were carried out with chromosome 9 because a number of linked genes are located there which influence kernel development in an easily observed manner. In the short arm of chromosome 9, the following order was established: terminal knob-*Yg*-*C*-*Sh*-*Wx*-centromere. *Yg* is a plant color gene; *C* affects pigment formation in the aleurone layer, *c* giving a colorless phenotype; *Sh* determines normal endosperm development, *sh* giving shrunken endosperm; *Wx* determines formation of normal starch which stains blue with iodine, and *wx* determines a mutant waxy form of starch which stains red with iodine.

Figure 4.2a shows a normal chromosome 9 and a rearranged chromosome obtained after X-irradiation. When these chromosomes pair at meiosis, one finds the kind of configuration shown diagrammatically in Figure 4.2b. The complicated configurations of rearranged chromosomes seen at meiotic prophase have provided some of the best cytological evidence that forces exist which determine a point-to-point localized pairing between homologous chromosomes in meiosis. When crossing over occurs between the paired chromosomes of Figure 4.2b, various types of aberrant products may result, depending upon the location of the exchange. In Figure 4.2c a dicentric (two-centromere) chromosome is shown, which was obtained as a result of a crossover somewhere in regions 1 to 5. When such a dicentric chromosome divides at the next mitosis, the two centromeres move to opposite poles of the mitotic spindle; the connecting bridge of chromosomal material is stretched and breaks, so that at each pole there is now a chromosome with a broken end. This broken end then initiates a breakage-fusion-bridge cycle, of the type shown in Figure 4.3. With the use of genes that are expressed in the endosperm, it is possible to follow the succession of breaks and consequent losses of particular dominant genes.

In further studies of the breakage-fusion-bridge cycle, it was shown that broken ends fuse 2-by-2 in endosperm tissue, but not in developing plant tissue. There they heal, and if the chromosomal rearrangement is not lethal, it remains stabilized as such. In general, in the endosperm, fusion of broken ends occurs even if one broken end is introduced by one parental nucleus and the second end comes from the other parent. The mechanisms of healing and of fusion have not been established, but the thorough analysis of the behavior of broken ends carried out by

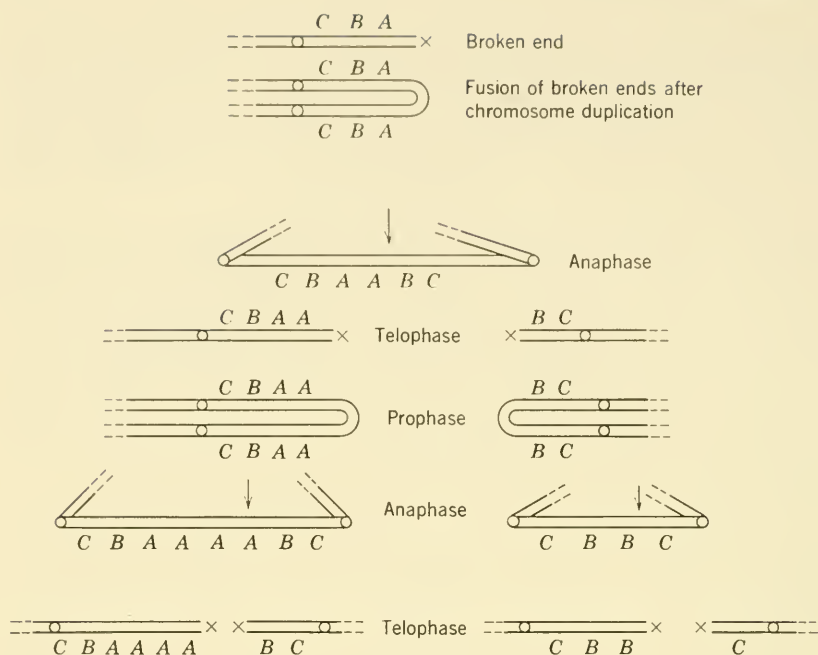


FIGURE 4.3. The chromatid type of breakage-fusion-bridge cycle. An illustration of the method by which variegation may be produced in tissues carrying a chromosome with a broken end. The dominant genes A, B, and C are carried by the arm with the broken end. The homologue of this chromosome (not diagrammed) is considered to be normal and to carry the genes a, b, and c. Duplication of this broken chromosome results in fusion at the ends, giving rise to a dicentric chromosome which breaks at the following anaphase. The arrow points to the position of breakage; the two broken chromosomes enter sister telephase nuclei. This process is repeated in successive mitoses, leading to deletion or duplication of segments of genetic material in particular nuclei (after B. McClintock, 1941, *Genetics* 26:234).

McClintock provides a valuable foundation upon which further studies can be constructed.

Aberrations resulting from X-irradiation, ring chromosomes, and the breakage-fusion-bridge cycle have provided some of the best material for studying the effects of deletions and duplications of bits of chromosomal material upon phenotypic expression of particular genes and upon viability. Many recessive alleles seem to exert no influence upon the phenotype, but only a comparison between recessivity and deficiency can provide critical evidence on this point. At the eye-color locus,

w, in *Drosophila*, for example, the w^0 allele differs from a deficiency for the locus only by its ability to back-mutate towards wild-type expression. Such an allele, totally lacking in expression, is called an *amorph*. Recessive alleles which produce a diminished expression, e.g., a pale pigmentation, are called *hypomorphs*.

Studies of gene dosage have also provided evidence of hypomorphism. For example, in two recessive mutants of *Drosophila*, bobbed and shaven, increasing the number of recessive alleles (possible because these genes are carried on the Y chromosome which can be present three times without lethal effect), leads towards the expression of the wild-type phenotype. Apparently each allele contributes a small positive phenotypic effect, undetectable when present only once, but cumulative at higher doses.

Small nonlethal deficiencies have been very useful for the cytological localization of particular genes. A heterozygous deficiency appears as an unpaired loop in the paired chromosomes of meiotic prophase of *Drosophila* salivaries, and, consequently, very small deletions can be detected. When the deletion includes a gene of known function, its location can be determined with considerable precision on the cytological map.

Duplications of chromosomal segments are less apt to be lethal than are deficiencies. In salivary gland chromosomes of dipteran insects, duplications have been identified as *repeats*, short segments which precisely repeat the banding pattern found elsewhere, often in an adjacent location. It has been suggested that repeats provide genetic material for progressive evolution; since the organism does not need to have the same information present twice, one of the repeats is available for mutation.

This suggestion is supported by the existence of closely linked genes with similar but not identical functions. In most organisms, such instances are found but rarely. In the bacteria, however, the close linkage of genes with related functions in a biosynthetic sequence has been found especially in *Salmonella* (see Chapter 6). An example of a repeat that has been observed and studied is *Bar* in *Drosophila*. Sturtevant studied this locus and inferred from genetic data alone that what had been called mutation at this locus was actually the result of oblique pairing and unequal crossing over, leading to the formation of repeats. It only became possible to test this inference by direct cytological examination some ten years later, and at that time, the correlation found between the *Bar* phenotypes and the presence of cytologically observable repeats fully supported and confirmed Sturtevant's hypothesis.

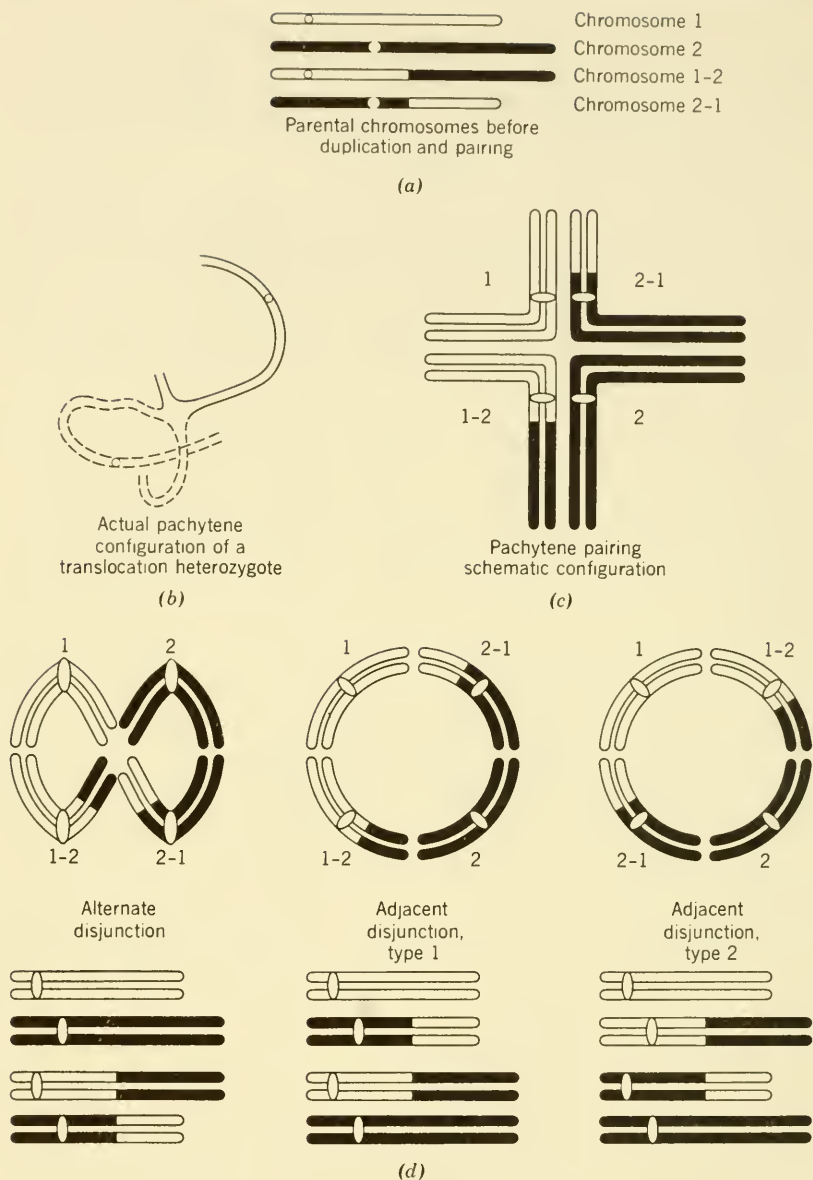


FIGURE 4.4. Translocation heterozygote paired at pachytene.

(a). Parental chromosomes are shown before duplication and pairing in meiotic prophase. In the reciprocal translocation between chromosomes 1 and 2, each chromosome is numbered with the sector containing the centromere given first.

(b). An actual pachytene configuration redrawn from a photograph shows pairing

Translocations

A translocation is a transfer of a chromosomal segment to a non-homologous chromosome. Most viable translocations detected after X-ray involve reciprocal exchanges between two nonhomologous chromosomes. The first identification of a translocation was by Bridges in 1919 in *Drosophila* on the basis of anomalous breeding data, indicating linkage between markers which he knew to be on nonhomologous chromosomes. Subsequently, translocations have been detected frequently in natural populations, particularly in plants. In the 1930's, translocations came into widespread use in genetic analysis when methods were established for selecting desired combinations from large populations of irradiated organisms.

Heterozygous translocations give rise to special pairing configurations in meiosis, as shown in Figure 4.4. The extent of lethality among the progeny depends upon whether or not the daughter cell receives a full chromosome complement. In plants, lethality is usually expressed immediately as pollen sterility, which is detected as shriveling of pollen grains, and provides a simple basis for identifying translocations in breeding experiments. The point of translocation can be mapped like a gene; and, with suitably marked stocks, four-armed linkage maps are found.

Inversions

An inversion is a chromosomal rearrangement in which a segment is reversed relative to the standard arrangement in the wild type. If the inverted segment is confined to one arm of the chromosome, it is called *paracentric*; if it extends across the centromere, it is called *pericentric*.

of the reciprocal translocation between maize chromosomes 8 and 10. Each duplicated chromosome is shown as a single line (after Rhoades, 1955, *The cytogenetics of maize*; in: *Corn and Corn Improvement*, New York, Academic Press).

(c). Schematic model of pachytene configuration of the chromosomes shown in a after duplication and pairing. Clear ovals refer to centromeres which are not yet divided. Crossovers occurring distal to the centromere result in terminal association of chromosome ends after contraction of chromosomes and movement onto the metaphase plate.

(d). Three orientations of translocation configurations on the metaphase plate, showing the genetic consequences of each. Alternate disjunction results in viable progeny, since both normal chromosomes go to one pole and reciprocally translocated chromosomes to the other pole. Both types of adjacent disjunction give rise entirely to lethal progeny since none of the gametes receive a full complement of genetic material.

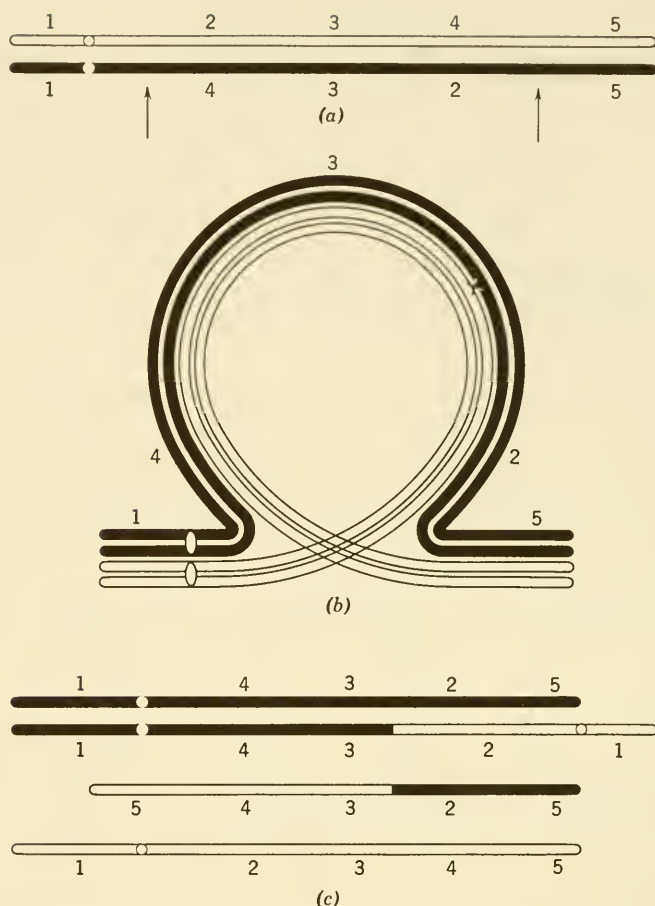


FIGURE 4.5. Inversion heterozygote paired at pachytene.

(a). The normal chromosome and its homologue with an inverted segment are shown before duplication and meiotic pairing.

(b). The configuration resulting from pairing of the homologous chromosomes of (a) consists of an inversion loop. The progeny are viable so long as crossing over does not occur within the loop. When it does occur, for example, between regions 2 and 3, the results shown in (c) are obtained.

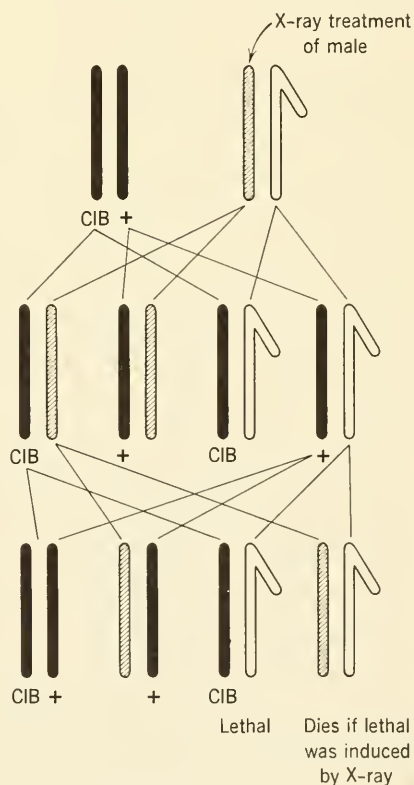
(c). Consequences of crossing over within an inversion loop. If a crossover occurs in the region between 2 and 3, the result will be the four chromosomes shown here. Two are parental, and will give rise to viable offspring. The other two will give rise to lethal progeny, since one chromosome is dicentric and will break in subsequent divisions, and the other product is an acentric chromosome which will be lost.

The presence of a heterozygous inversion is characteristically seen as an inversion loop in paired homologous chromosomes, as shown in Figure 4.5. If no crossover occurs within the loop, there are no aberrant genetic or cytological consequences. If a crossover does occur, there are definite consequences.

The cytogenetic consequences of crossing over within a paracentric inversion loop are shown in Figure 4.5c. The noncrossover strands are unaltered, but both crossover chromatids are lost: one because it lacks a centromere, cannot orient on the spindle, and is eliminated; and the other because it has two centromeres which move to opposite poles producing an anaphase bridge which finally breaks at random, giving rise to two incomplete chromatids. This effective elimination of crossover chromatids results in the apparent absence of crossing over. Thus inversions were originally detected as crossover suppressors and were utilized as such for a long time before their cytological basis was established.

The most celebrated use of a crossover suppressor was in Muller's famous CIB stock of *Drosophila*, which he developed for the detection

FIGURE 4.6. The CIB method of Muller. The chromosome marked CIB contains a long inversion, a dominant mutant gene *B* (not present on the Y chromosome) and a recessive lethal, *l*. X-rayed males are mated with CIB females, and in the F_1 generation, only CIB females are saved. Each is mated individually and the progeny scored for the presence of males. If no males are present, it may be inferred that a lethal was induced in the irradiated X chromosome (after Sturtevant and Beadle, 1939, *An Introduction to Genetics*, Philadelphia, Saunders, p. 209).



of recessive lethals induced by mutagenic treatment. Muller's demonstration of the mutagenic effects of X-ray in 1927 required a reliable screening procedure to detect and count rare mutations; the CLB method as shown in Figure 4.6 is just that.

Paracentric inversions are commonly found in natural populations of plants and of the dipteran insects; they have been looked for in few other forms. Because the presence of heterozygous inversions results in the suppression of recombination within the inverted segment, it may be supposed that consequences of evolutionary interest might result. A long and fascinating investigation of inversion patterns in many species of *Drosophila* in relation to their ecological habitats, by Dobzhansky and co-workers, has provided a wealth of information about the evolutionary role of inversions in this genus. There is no doubt that the locking together of certain gene combinations within inverted chromosomal segments has great selective advantages. There seems even to be an optimum inversion length, long enough for the accumulation of new mutations, but short enough so that few viable double crossovers will occur.

VARIATIONS IN CHROMOSOME NUMBER

Polyploidy of Organisms

Integral number increases in the chromosome complement are of widespread occurrence in natural populations, particularly of plants, in which polyploidy represents a popular evolutionary device. Polyploidy has played an important role in the improvement of agricultural plants, both by the selection of more favorable strains, subsequently found to be polyploid, as in the evolution of domestic wheat, and in planned breeding programs.

For example, a rare hybridization between distantly related strains can sometimes be stabilized by a subsequent doubling of the entire complement, as shown in Figure 4.7. The homologous chromosomes from the rather different original parents no longer pair in meiosis; and pairing is limited to the closely related homologues of each parental complement. Thus, regularity of meiosis and fertility of the new hybrid, called an *amphidiploid*, are assured.

In general, polyploid plants are far more common than polyploid animals. Perhaps the mechanism of sex determination in animals, which is based upon the segregation of sex chromosomes and balance in number with the autosomes, cannot readily be applied to accidental polyploidy.

Polyploidy of Cells and Tissues

Within organisms of a particular ploidy, multiplication of the chromosome complement is widely found in particular cells and tissues. For example, in the roots of diploid plants, the cells behind the growing

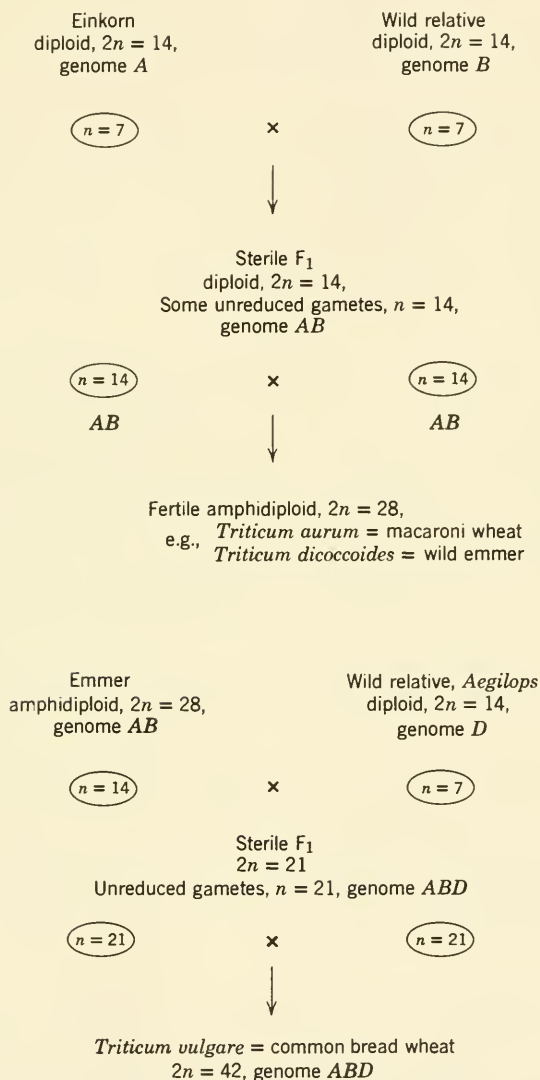


FIGURE 4.7. Origin of common bread wheat, illustrating the method of evolution by amphidiploidy.

point are tetraploid, and further back they become octoploid. Perhaps the most celebrated example is that of the polytene chromosomes of some insect larvae, in which each chromatid is present as many as 1024 times. This type of polyploidy, in which the strands multiply but do not separate, is called *polyteny*. The term *endomitosis* has been applied to the situation in which duplication is followed by separation of the strands within a single nucleus.

Aneuploidy

Aneuploid cells contain odd numbers of chromosomes, other than integrals of the haploid number. Trisomics, for instance, contain $2n + 1$ chromosomes, monosomics, $2n - 1$. In general, aneuploid organisms are rare, although trisomic plants are occasionally found in nature. Within organisms, the occurrence of aneuploid cells in normal tissues is also rare, as judged from chromosome counts and cytochemical measurements.

Aneuploidy seems to be an important characteristic of malignant tumors, and, consequently, chromosome constitution has become of great interest in the study of cancer. Some investigators have proposed that the development of malignancy involves increasing genotypic variability of individual cells so that selection can operate to favor those capable of multiplying rapidly within the host organism. It is further proposed that one method of increasing variability is by altering the chromosome complement. Most malignant cell lines which have been examined are hypotetraploid; thus, it is the presence of additional chromosomes above the diploid number which is significant.

Recent chromosome studies of normal mouse cells immediately after transfer to tissue culture have shown that explantation alone often leads rapidly to the development of aneuploidy. First the tetraploid number becomes predominant, and then fast growing sublines take over the culture; they are hypotetraploids of various sorts. These findings are important not only in the investigation of cancer but also for the light they cast upon experimental difficulties involved in the study of tissue-cultured cells.

CROSSING OVER

The term crossing over refers to reciprocal genetic exchange between homologous chromosomes.

Each chromosome of meiotic prophase consists of four chromatids, two

being the daughter chromatids of each parental homologue. This association, called a *bivalent*, results from synapsis between the homologous chromosomes, which are already double. As the chromosomes thicken and shorten by coiling and begin to separate, it appears that exchanges have occurred between chromatids. Under the microscope one sees *chiasmata*, so called because they confer upon the separating chromosomes a cross-shaped appearance.

The most direct evidence that crossing over occurs at the four-strand stage, when all four chromatids are together, comes from tetrad analysis. (see Chapter 3, p. 73). If a crossover occurs between two linked markers, then four genetically different progeny are recovered, two parental and two recombinant. In the development of genetics, the occurrence of crossing over at the four-strand stage was established with great difficulty in organisms in which only a single product, rather than all four, is normally recovered from each meiosis. Special *Drosophila* stocks, similar to the nondisjunctional strain discovered by Bridges (p. 91), in which two X chromosomes are passed from mother to female offspring, provided the basis for what has been called half-tetrad analysis. With this material, some details of the crossing-over process were investigated.

The principal regularities established in cytogenetic studies of crossing over were that: (1) genetic crossing over involves the physical exchange between segments of homologous chromosomes; (2) the occurrence of genetic crossing over correlates well with the frequency of cytologically observable chiasmata; and (3) there is an apparent randomness in choice of nonsister chromatids which exchange with one another in any one crossover event. Whether there is also exchange occurring between sister chromatids in meiosis is as yet an unresolved question.

Correlation of Genetic Crossing Over with Physical Exchange

This correlation was established by Stern with *Drosophila*, and by Creighton and McClintock with maize, who utilized chromosomes heterozygous for cytological as well as genetic markers. The critical crosses are shown in Figure 4.8; both systems represent classical examples of the clarity and beauty of cytogenetic experiments.

Correlation of Crossing Over with Chiasma Formation

Janssen first proposed, in 1909, that each cytologically observable chiasma was the result of an exchange between homologous chromosomes, with the genetic consequence of recombining linked markers.

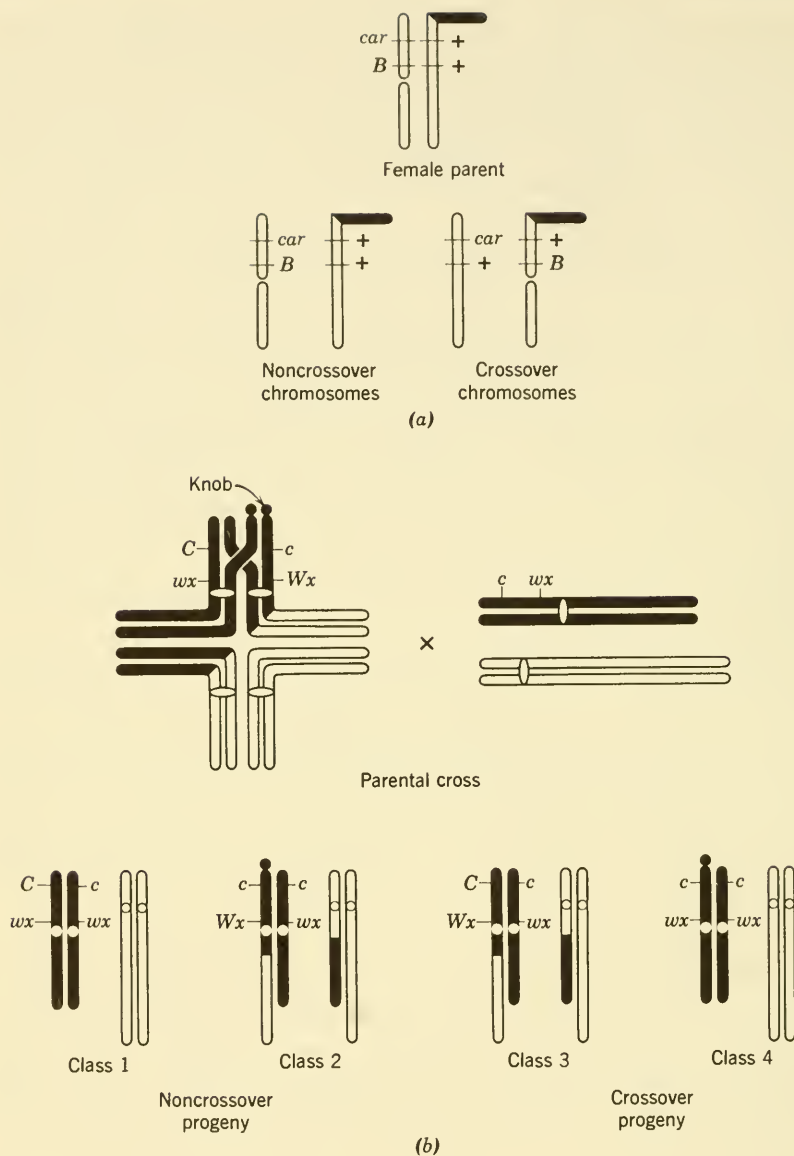


FIGURE 4.8. The correlation of cytological exchange with genetic recombination: a demonstration that physical exchange between chromosomes is involved in crossing over.

(a). *Drosophila* experiment in which the X chromosomes of the female parent are labeled both genetically and cytologically. Examination of progeny flies exhibiting genetic recombination between *car* and *B* showed that *car*⁺ flies had neither the

Slowly this chiasmotype theory became generally accepted, and until recently most cytogeneticists agreed that every chiasma represented a genetic crossover, and that there were no chiasmata without crossing over. The observation by Cooper of chiasmata in the male *Drosophila*, in which there is no genetic crossing over, has cast some doubt upon the generality of Janssens' theory. On the other hand, there is fairly good quantitative evidence indicating that the frequency of chiasmata parallels the frequency of crossing over found in maize. Interlocking rings and other special configurations found with aberrant chromosome pairing also support the chiasmotype theory. The present situation is thus an unsatisfactory one: no fully acceptable hypothesis has been proposed to account for the occurrence of chiasmata.

Chiasma Interference

The frequency of recombination between closely linked markers is often found to be lower than expected on the basis of linkage with more distant markers. This phenomenon has been attributed to interference of one chiasma in the formation of a neighboring second chiasma. In actuality, the term interference has only a formal significance, since the mechanism is not known. With very closely linked markers, an increase in the frequency of recombination above that expected has been observed and given the logically correct but clumsy title of negative interference.

Tetrad analysis is particularly well suited for the quantitative estimation of interference, and studies with several microorganisms are being carried out to investigate the process of interference in relation to the mechanism of crossing over as a whole.

Chromatid Interference and Sister-Strand Exchange

If an investigation is made of the recombination of linked markers situated some convenient distance, say 5 to 10 map units apart, one finds that exchanges are always reciprocal, with the recovery of all the input

break nor the translocated segment of the parental X chromosomes while +B flies had both (adapted from C. Stern, 1931, *Biol. Zbl.*, 51:547).

(b). Maize experiment in which chromosome 9 was labeled both genetically and cytologically. Noncrossover progeny had the same chromosomes as did the parents, while C Wx progeny contained the translocation but not the knob, and c wx progeny contained one knobbed chromosome but no translocation. Thus the exchange must have occurred between C and Wx as diagrammed (adapted from Creighton and McClintock, 1931, *Proc. Natl. Acad. Sci. Wash.* 17:492).

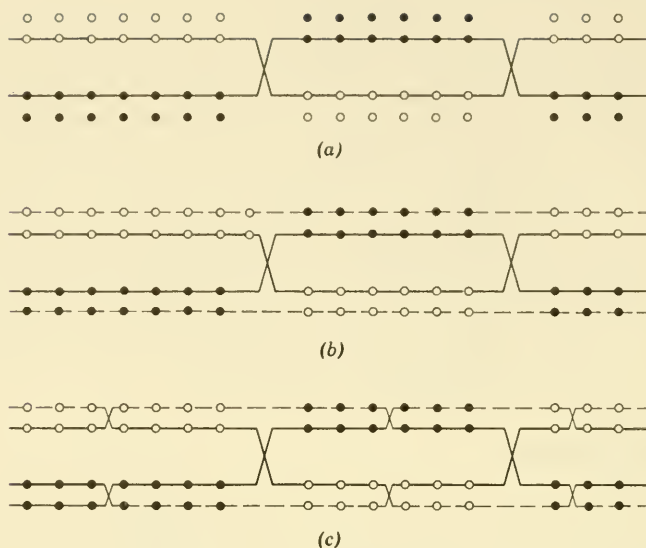


FIGURE 4.9. Diagram of Belling's hypothesis of chromosome duplication, plus the added effects of sister-strand crossing over.

(a). The linked series of open dots indicate one chromosome (double helix) and the black dots indicate a paired homologous chromosome. According to Belling, the dots represented genes; on a more modern view, consider the dots as nucleotide pairs. The parental chromosomes are twisted about each other, and new strands form in association partly with one homologue and partly with the other.

(b). Consequences of this model: only two of the four chromatids can be recombinant. To obtain recombination among all four strands an additional step of sister-strand crossing over is postulated.

(c). An example of the effects of sister-strand crossing over imposed upon Belling's model.

(a and b after Swanson, 1957, *Cytology and Cytogenetics*, Englewood Cliffs, N. J., Prentice-Hall, p. 288).

markers in a functionally unchanged condition. In fact, the reliability of these results provided the great predictive strength of classical genetics. Subsequently, using half-tetrads in *Drosophila*, it was shown that not only did single crossovers occur with a fairly constant frequency between any two genes but also that two-, three- and four-strand double crossovers occurred with the frequencies predicted on the basis of randomness of exchange between any two of the four chromatids. The latter result was referred to in the literature as the "absence of chromatid interference" in crossing over. Unfortunately, the results obtained are not really conclusive because the interpretation involves the assumption that sister-strand crossing over does not occur.

In 1931, Belling proposed that crossing over occurred at the time of

chromosome duplication. If this were correct, it would seem that only the new strands could be involved in an exchange. Consequently, with the demonstration that all four strands take part, Belling's hypothesis was dropped. However, if one invokes the occurrence of sister-strand crossing over, then Belling's hypothesis can be reconsidered, as shown in Figure 4.9. There is at present no critical evidence either establishing or excluding the occurrence of sister-strand exchange at meiosis. In a recent re-evaluation of crossing over based upon all the available genetic data in *Drosophila melanogaster*, Weinstein has concluded that either sister-strand crossing over does not occur or, if it does occur, that it does not interfere with nonsister exchanges between homologous chromosomes. This conclusion does not permit a decision with respect to the Belling hypothesis.

As the result of new studies of chromosome duplication, utilizing radioactive tracers to distinguish between parental and newly formed strands, it is becoming possible to reinvestigate crossing-over mechanisms at the molecular level. New genetic data, obtained by mapping very short regions, indicate that some exchanges may occur by a process very different from that envisaged from studies of recombination over larger intervals. These problems will be reconsidered in Chapter 7, after presenting some recent information on chromosome duplication and fine-structure mapping.

Physiological Influences upon Crossing Over

Before closing the discussion of classical studies of crossing over, it should be noted that crossing over is neither an invariant process occurring at all meioses nor a random process insensitive to environmental influences. No crossing over occurs at meiosis in the *Drosophila* male which is XY, nor in the silkworm female, which is XO. In general, in those organisms in which differences in crossover frequency have been observed between the sexes, it is the XY or XO sex in which crossing over is reduced or absent. Such factors as age, nutritional condition, and temperature alter crossover frequencies considerably in *Drosophila*, but not all regions are uniformly affected. Those near the centromere are most susceptible.

These varied facts, not at all understood, are of interest for two reasons: Any theory of crossing over must eventually explain them; and, conversely, they may provide some clues or leads in the investigation of crossing-over mechanisms.

In summary, this chapter has cited some of the classical evidence which established the chromosomal location of genetic determinants,

by correlating observations from the very different disciplines of genetics and cytology. The segregation and recombination of genes were found to be governed by the mechanics of chromosome behavior, whether normal or aberrant. The recombination of linked genes, attributed to the process of crossing over, was shown to exhibit certain regularities: recombination at the four-strand stage, reciprocal recombination in each individual meiosis, correlation of recombination frequency with chiasma frequency, positive chiasma interference. These regularities, generalized as the partial chiasmatype theory, are characteristically found in studies of linked genes, several map units apart, undergoing recombination during meiosis.

BIBLIOGRAPHY

- Belling, J., 1933. Crossing over and gene rearrangement in flowering plants. *Genetics*, **18**:388-411.
- Creighton, H. B., and B. McClintock, 1931. A correlation of cytological and genetical crossing over in *Zea mays*. *Proc. Natl. Acad. Sci. Wash.*, **17**:492-497.
- Mangelsdorf, P. C., 1953. Wheat. *Sci. Amer.* **189**:50-59.
- McClintock, B., 1941. Spontaneous alterations in chromosome size and form in *Zea mays*. *Cold Spring Harbor Symp. Quant. Biol.*, **9**:72-81.
- Rhoades, M. M., 1955. The cytogenetics of maize. In: Sprague, G. F. (ed.), *Corn and Corn Improvement*. New York, Academic Press, pp. 123-219.
- Stern, C., 1931. Zytologisch-genetische Untersuchungen als Beweise für die Morgan'sche Theories des Faktorenaustauschs. *Biol. Zbl.*, **51**:547-587.
- Sturtevant, A. H., and G. W. Beadle, 1939. *An Introduction to Genetics*. Philadelphia, W. B. Saunders (Chapters 1, 2, 7, 8, 9, 10, 11), 291 pp.
- Swanson, C. P., 1957. *Cytology and Cytogenetics*. Englewood Cliffs, N. J., Prentice-Hall (Chapters 4, 6, 8, 10), 596 pp.

5 Recombination in Viruses and Bacteria

We are accustomed to think that mating must somehow involve sex. Such a concept comes naturally out of our own experience. During the early development of genetics, it was not only assumed that sex was necessary for genetic analysis but also that the recombination of genes necessarily depended upon the fertilization of eggs by sperm. In recent years, novel recombination processes have been discovered which are probably of widespread occurrence, not only among microorganisms but also in the somatic cells of higher organisms including man.

Before discussing these processes, something will be said about sex and sexual reproduction. The classical definition of sex is morphological. The dictionary says that it is the character of being either male or female. Among animals sex is obvious because of the different structure and habit of male and female individuals. Plants too are sexual. In their reproduction, the male and female gametes which fuse together in fertilization are the products of different meioses.

From the point of view of genetics and evolution, however, the essential feature of sex is that it makes possible the recombination of genes from different parents. In this way, new assemblages of genes arise which then become available for the selective action of the environment.

Some microorganisms such as *Neurospora* are sexual but hermaphroditic; usually all strains can act both as the female parent by forming fruiting bodies and as the male by fertilizing the fruiting bodies of another individual. The zygote undergoes a true meiosis and the products of a *single* meiosis may unite (see Figure 2.9). Some microorganisms such as *Chlamydomonas* and yeast do not even have a structure such as the fruiting body which can be compared to the egg. The cells of these forms fuse together in a process called *syngamy* which leads to the pro-

duction of diploid zygote nuclei which unite two complete genotypes. In this regard, and because meiosis sooner or later ensues, syngamy and sexual reproduction are similar.

With the advent of more extensive investigations into the genetics of microbes, other mechanisms of mating have been revealed. Some involve the incomplete transfer of the genotype of the donor individual, and the recipient has more than one, but less than two, sets of genes. In such partial zygotes meiosis, and even mitosis, may not occur; the partitioning of genetic material is apparently some simpler consequence of the replication of DNA.

MATING AMONG VIRUSES

Even viruses are able to exchange their genes. To ask whether these minute structures are living or not is a question of semantics. Whatever the answer, when they are inside host cells they behave like many other cell particulates, influence the biochemistry of the host, and multiply. Such extensive information about the biochemistry of viral infection has been obtained that it has been possible to relate genetic to chemical events. The term *molecular biology* is not inappropriate when applied to many investigations in this field.

For reasons of experimental convenience, the most detailed studies of host-virus relations have been conducted with bacterial viruses, notably the *T* phages which attack *E. coli B* (see Chapter 1). They are called bacteriophages because they destroy their bacterial host. When a dilute phage suspension is spread on a thin film of sensitive bacteria growing on an agar plate, each phage particle will adsorb on one bacterium and multiply within it until the cell breaks down (*lyses*) and releases newly formed phages. These, in turn, infect adjacent cells, and the process continues until a visible area on the plate has been cleared of bacteria. Such a spot is called a *plaque* (Figure 3.8), which is the usual unit of observation in experiments with phages. The appearance of the plaque (turbidity, margin, etc.), and its size depend upon phage genes which can mutate.

When a virulent phage adsorbs on the surface of a sensitive bacterium, the synthetic reactions of the host cell which were going on before infection are blocked; respiratory metabolism is arrested in the state existing at that moment, and it is no longer possible to elicit the formation of adaptive enzymes. The formation of new protein begins and there is a turnover of RNA which, as it is degraded, is replaced by a new type. DNA synthesis ceases completely (Figure 5.1). As a matter of fact, the

pre-existing bacterial DNA is degraded and its breakdown products enter a pool to which contributions are also made from the surrounding medium. From this pool, constituents are withdrawn for the DNA synthesis which soon begins again, now under the control of the virus. The virus-initiated synthesis of new protein, which consists of many different species of molecules, is a pre-requisite for this DNA synthesis, as is shown by experiments in which the drug, chloramphenicol, is used to prevent the formation of protein. When, as is sometimes the case, the virus DNA is unique in containing hydroxy-methyl-cytosine instead of cytosine, the host is caused to make this unaccustomed base. Even if the host is a mutant unable to synthesize thymine, the infecting phage DNA has the information to carry out this synthesis which is needed for its own multiplication. Thus the viral DNA behaves throughout as a parasite; its genes replace those of the bacterium, and the metabolic machinery of the host cell is re-directed toward making phages.

The time required for phage reproduction in a single bacterium is short; after a period of often less than 30 minutes, the infected bacteria burst and release some hundred newly formed phage particles. This time is called the latent period. When the infected cell is opened artificially during the first half of this period, no phages are found, not even the original infecting one. It has, of course, been dissociated, its protein remaining outside the cell after its DNA was injected. This DNA, called *vegetative phage*, multiplies during the early latent period and

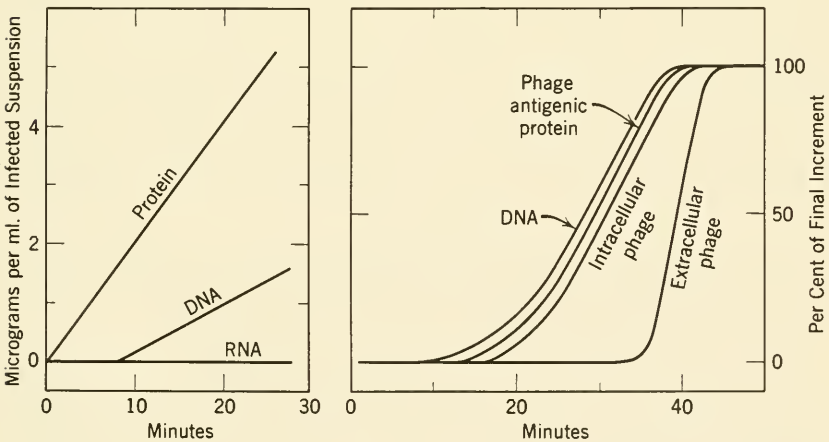


FIGURE 5.1. Some biochemical syntheses that are involved in the multiplication of bacteriophages (after Cohen, in Luria, 1953, *General Virology*, p. 178, New York, Wiley).

is present as fibers not encased in a protein coat. It is clear that these fibers, which contain phage genes, multiply exponentially as though each newly formed one acts as a template for the formation of another. We draw this conclusion from the fact that the number of phage mutants formed in different cells is distributed according to the calculations of Lea and Coulson, which assume the production in series of two particles from one (see Table 2.1). During the last part of the latent period, mature infectious phages are assembled from the separately multiplying DNA, protein coats and tails. By prematurely disrupting the cell, these intracellular phages can be found to increase arithmetically as though during maturation the pool of exponentially multiplying DNA were tapped by some process occurring at a constant rate. The DNA that gets into the newly formed phages cannot replicate and, as a consequence, the over-all rate of increase of DNA in an infected cell becomes arithmetic.

It is most interesting that the bulk of this phage DNA seems to pass intact from generation to generation. Levinthal used an autoradiographic technique to demonstrate this. Phage DNA was labeled with P^{32} (by growth on P^{32} -grown bacteria) and separated by osmotic shock from the phage protein fractions. Both intact and disrupted preparations were plated in a photographic emulsion where the P^{32} was allowed to disintegrate before the film was developed. Each piece of radioactive DNA was a focus for the emission of β rays, detected by the microscopic observation of tracks of exposed silver grains caused by the passage of fast electrons. The more P^{32} in a DNA particle, the more disintegrations during the exposure period, and for every disintegration there is one ionization track. Therefore, if 25 disintegrations occurred in a piece of DNA, a star pattern with 25 arms would result in the film (Figure 5.2).

It was observed that the DNA released from the phage coat formed stars with about 40 per cent of the number of arms found when intact P^{32} -labeled phages were plated in the emulsion (Table 5.1). Therefore,

TABLE 5.1

Number of P^{32} Atoms in the Star-Forming Particles Relative to the Number in the Intact 72 Phages which is Corrected to 100

(From Levinthal, 1957, in McElroy, and Glass *The Chemical Basis of Heredity*. Baltimore: The Johns Hopkins Press, p. 737).

	Intact Phage Particles	After Osmotic Shock
Uniformly labeled parental phages	100	40 ± 4
1st-Cycle progeny	24 ± 3	23 ± 3
2nd-Cycle progeny	26 ± 3	too dilute for study

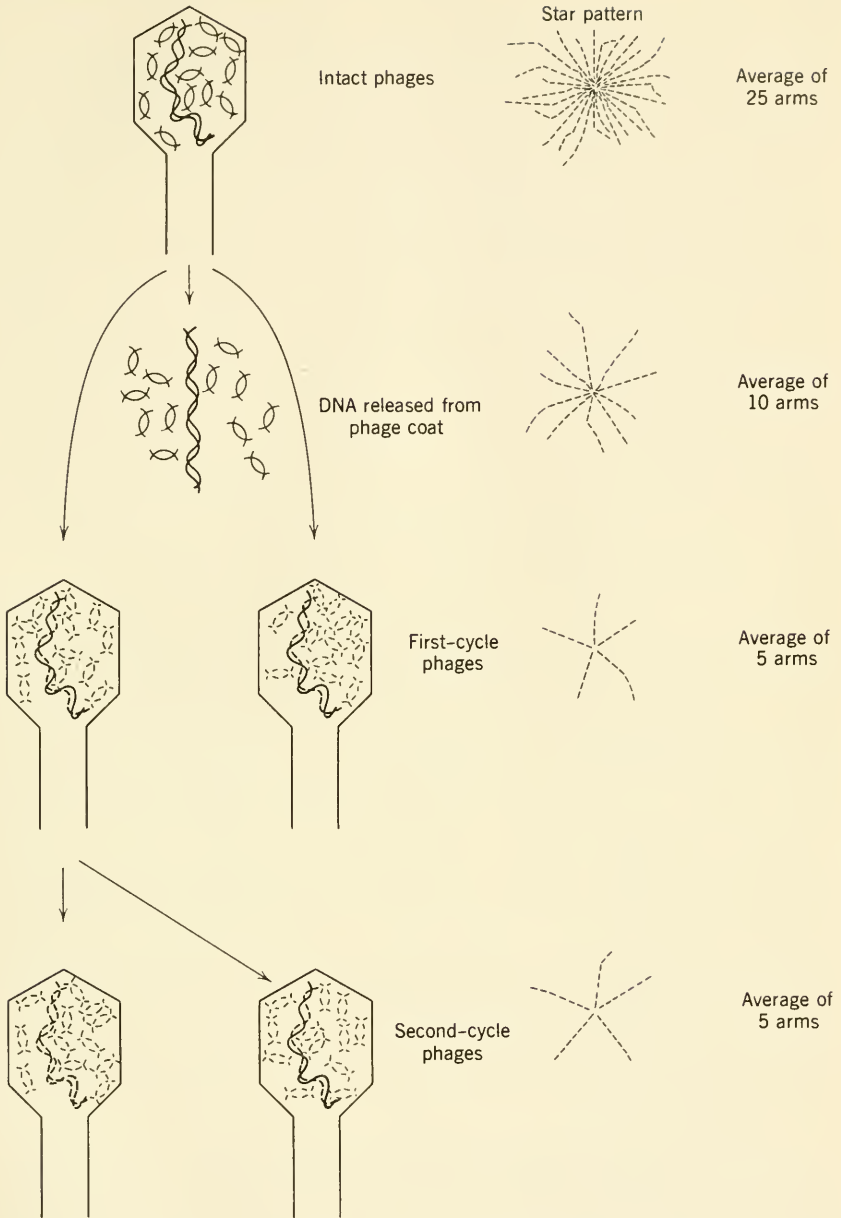


FIGURE 5.2. Diagram of the results of the star experiment which traces DNA during phage development (after Levinthal, 1957, in: McElroy and Glass, *The Chemical Basis of Heredity*, p. 737, Baltimore, The Johns Hopkins Press).^{*}

^{*}Recent evidence (Rubenstein, Thomas & Hershey, 1961, Proc. Nat. Acad. Sci., Wash., 47:1113), which shows that the DNA in bacteriophage is a single intact molecule, requires that this experiment be reinterpreted.

about 40 per cent of the DNA remained in the form of large molecules; the rest was present after release in very small pieces. These small pieces have an unknown function, but the large piece clearly passed from one generation to the next. After a cycle of infection, the stars formed by the progeny had some 20 per cent as many arms as the stars formed by the original infecting DNA particle; the number remained constant at this level after the next cycle. These observations are consistent with the notion that the infecting DNA contains a large molecule making up about 40 per cent of the total. This molecule may be in the form of a double helix, replicating according to the Watson-Crick model. Thus the first cycle phages have 20 per cent of the total radioactivity and, since the two strands of the double helix each remain intact, all future cycles yield progeny with the same amount of radioactivity. These results are comparable to those found with the chromosomes of other organisms (Chapter 7), and indicate not only a doubleness of the genetic material but also a semiconservation of its organization. That the large DNA molecule is genetic material is demonstrated by the fact that at least some genetic markers pass with it from cycle to cycle.

With this information in mind we may turn our attention to the recombination that occurs when different phage mutants multiply in the same cell. If two related strains of phages, say of genotypes *AB* and *ab*, are mixed in excess with a suspension of sensitive bacteria, nearly every bacterium will be infected by both types. The phage progeny of such a cross will be of four genotypes: *AB*, *ab*, *Ab*, and *aB*. The reciprocal cross, *Ab* $\times$ *aB*, gives the same kinds of progeny, but this time the recombinants are *AB* and *ab*; they appear with the same frequency as did *Ab* and *aB* in the first cross. Thus the coupling of genes in the parents does not determine the frequency of recombinants; the particular genes involved in the cross determine the rate of exchange. Evidently a linkage of genes exists in phages just as in other organisms; the frequency of recombination between different genes is roughly additive, and linear genetic maps have been constructed (Table 5.2). When closely linked genes are studied, however, evidence for negative interference is obtained: the occurrence of one genetic exchange increases the likelihood of an adjacent one.

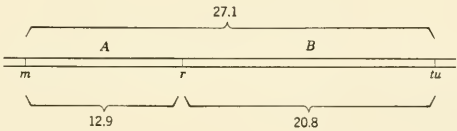
It must be appreciated that the progeny of a phage cross do not result from one generation of multiplication. No matter how few phages infect a cell, some hundred progeny may be recovered. Several generations of multiplication take place within each cell, and the multiplying phages have a chance to mate again and again. When many phages infect a single cell, there is no assurance that each one of them will mate or, conversely, that all progeny will result from a mating and not just from

clonal reproduction. Therefore, unlinked genes and those 50 or more units from one another may give recombinations between them in a frequency of less than 50 per cent; some of the old combinations come from unmated phages and are counted along with those from matings, although, of course, they should not be. The more phages that are formed in a single cell, the closer the frequency of recombinations between distant genes approaches 50 per cent, because as reproduction continues the chance increases that every phage will have had a mating in its ancestry. The figure of 50 per cent recombination for freely recombining genes can be obtained at will if we take advantage of a three-factor cross. By considering only the progeny that show recombination between two genes, we select viruses whose ancestors have mated. Among such progeny, the third gene shows 50 per cent recombination with any other with which it freely recombines.

When we make allowance for the fact that a phage cross constitutes a population experiment, many surprising results can be understood. For example, it then becomes clear how a single offspring can have three

TABLE 5.2
A Linkage of Genes in a Three-Factor Cross of T4 Bacteriophage in *E. coli*
 (From Doermann, 1953, *C.S.H. Symp. Quant. Biol.*, **18**:3)

Category	Region of Exchange	Genotype of Progeny	Total Phages of each Type	Per Cent
Parental		<i>m r tu</i>	3467	33.5
		<i>+++</i>	3729	36.1
Recombinant	A	<i>m ++</i>	520	5.0
		<i>+ r tu</i>	474	4.6
	B	<i>m r +</i>	853	8.2
		<i>++ tu</i>	965	9.3
	AB	<i>m + tu</i>	162	1.6
		<i>+ r +</i>	172	1.7



m = minute plaque mutant
r = sharp margin plaque mutant
tu = turbid plaque mutant

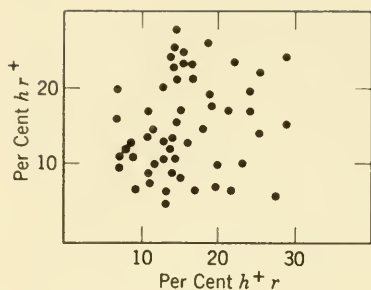


FIGURE 5.3. The lack of correlation between the numbers of reciprocal recombinants produced by individual bacteria, all of which were mixedly infected with two types of phages, $h^+ r^+$ and $h r$. The h mutant will infect a greater range of hosts than will the h^+ strain (from Hershey and Rotman, 1949, *Genetics*, 34:44).

parents when three types of phages infect the same cell. We can also understand how, when one parent forms a very small minority, the frequency of its genotype among the progeny may be smaller than the frequency of recombinants between it and the majority parent. The number of rounds of mating per cycle of multiplication in a bacterium can be calculated, and varies from 0.5 to 5 according to the phage.

But what is a "mating" between molecules of DNA? Is there a crossing over between the strands of duplicating DNA, or does the exchange occur in some other way? In the classic sense crossing over is a behavior of chromosomes, and it occurs in the four-strand stage, yielding reciprocal recombinants. When the progeny from large numbers of mixedly infected bacteria are collected as one assemblage, equal frequencies of reciprocal recombinants are found (Table 5.2). But when recombinants are recovered from singly infected bacteria, equal numbers of reciprocal recombinant types are not found (Figure 5.3). Sometimes, in fact, a cell will yield only one or the other of the two types of reciprocal recombinants. The equality observed when the products of many cells are pooled is due to randomness.

This lack of correlation can be explained by assuming that phage maturation consists of taking a small sample from a large pool of DNA particles for inclusion in protein coats. Thus, even though reciprocal recombinants may be formed by the recombination event, random sampling would lead to the formation of mature phages in which the frequencies of the reciprocal recombinants are scattered around a 1:1 ratio. Sometimes the population of phages formed in a single cell may even have included one but not the other reciprocal recombinant. But if this mechanism were operating, there should also be a low correlation between recombinants of the same type because the progeny of one recombinant will, on the average, be no more numerous than the progeny of its reciprocal. The facts contradict this line of thinking. In single bacterial bursts, if a recombinant of a particular type appears, there is a

high probability that there will be another of the same type; but the probability that the reciprocal type will come from the same cell is low. The prediction of a simultaneous reduction in self- and reciprocal correlation is not borne out. Therefore, reciprocal recombination products are not formed simultaneously by a single event.

Rather, all results are consistent with the partial-replica theory which accounts for recombination by another means than crossing over. This theory, which fits nicely with the Watson-Crick model of DNA, assumes that duplication of the genetic material occurs progressively along a template. On occasion a partial replica of the first template separates from it and is completed on another (Figure 5.4). Thus, if every new and old DNA molecule can act as a template, multiplication can be exponential, but every mating results in the formation of only one recombinant product. It is even possible that a single replica may copy more than two templates by a process of group mating. How this partial replica or copy-choice mechanism can be related to DNA structure will be discussed in Chapter 7.

In addition to accounting for the types of recombination that occur, the partial-replica hypothesis provides a model for a peculiar kind of heterozygous phage that is formed in crosses with a frequency of a few per cent. This phage is a single particle but it does not reproduce its kind. When it multiplies it gives rise to haploid phage containing one or the other genes for which it was heterozygous and, once again, to a few per cent of heterozygotes. The region of heterozygosity in the DNA molecule must be small because only when genes are closely linked are phages found which are heterozygous for both of them. When two distant genes are involved in a cross, the phages may be heterozygous for one of them. Upon reproduction two types are formed; one is an old combination and the other a recombination of the two genes. Figure

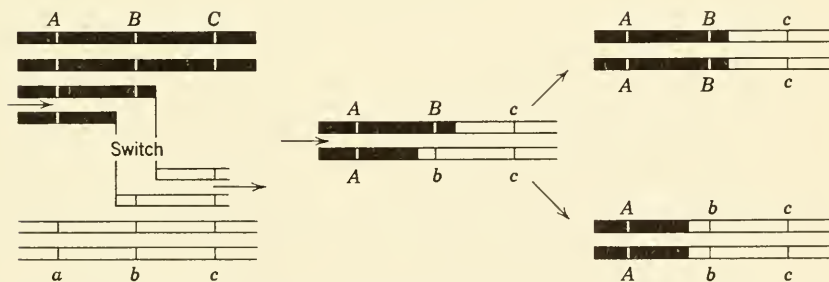


FIGURE 5.4. Diagram of a possible mode of partial replica formation and recombination in phages. See also Figure 7.12.

5.4 shows how this is possible and, in addition, accounts for the results of a three-factor cross in which some phages become heterozygous for the middle marker. In this case they are recombinant for the distal markers, as the model predicts. The structure and frequency of heterozygous phages are sufficient to account by their reproduction for all recombinants found.

There are other phenomena that have an explanation in the partial-replica hypothesis. For example, ultraviolet light increases the frequency of recombination in phages, probably by damaging some short region of the DNA molecule. When replication reaches that site it cannot progress. Unable to complete itself, the partial replica may leave its damaged template and finish its replication on another template, undamaged at the site in question. In this way some genes on a damaged phage may be "rescued" by replication in a recombinant particle. Genes in phages inactivated by P^{32} disintegration, which probably breaks the sugar-phosphate backbone of DNA by transmutation to S^{32} or through the recoil energy sustained by the decaying P^{32} nucleus, may be rescued in a similar way. This, and the fact that linked loci are lost more often together, is fine evidence that nucleic acids are actually the carriers of genetic information.

Clearly, phage genes are exchanged by a mechanism which can hardly be called sexual. There is no true zygote, and there is no evidence of processes like mitosis or meiosis. The phage DNA molecule, although not a nucleoprotein, is sometimes referred to as a chromosome because it comprises the assemblage of genes linked in a linear order.

LYSOGENIC BACTERIA AND PROPHAGE

The bacteriophages discussed thus far are called virulent because they invariably kill their hosts. Although the virulent phages have been studied most intensively, they are much less prevalent in nature than another class of bacteriophages, called temperate, which do not necessarily kill the bacteria they infect. The DNA of temperate phages may take up a symbiotic relationship with the bacterial host; in such an event the bacterium is not destroyed, and may even benefit from the presence of the phage. Bacteria which carry such phages are called *lysogenic*. When lysogenic bacteria are disrupted, no infectious phages are found within them. The noninfectious unit which is carried by lysogenic cells and which contains the information for phage formation is called *prophage*. During the growth of a lysogenic strain, some cells, at a rate that is usually from 10^{-2} to 10^{-5} per generation, lyse and release in-

fectious phages which they have formed (Figure 5.5). When temperate phages adsorb on sensitive bacteria, one of two results may follow: either the invaders may behave like virulent phages, multiply, and eventually lyse the cell; or instead, the invading DNA may be reduced to the prophage condition, multiplying innocuously in tempo with the host. This equilibrium is rarely broken spontaneously, but it can be disrupted in most cells by the application of inductive stimuli such as radiation or chemical mutagens and carcinogens.

Although the adsorption of a temperate phage clearly does not kill the host bacterium, the growth of a phage out of synchrony with a bacterium does. In this case the prophage is converted to a vegetative condition in which it multiplies, eventually becoming enclosed in a protein coat equipped with a tail. These mature, infectious, temperate phages are released by lysis of the host.

During the vegetative growth of two related strains of temperate phages, gene exchange may occur. This process has all the properties described for virulent phages. Recombination may even take place between a vegetative phage and a prophage, or between two prophages in the same lysogenic bacterium. Just how this recombination takes place is yet to be discovered. Of even more interest is evidence which indicates that some temperate or nearly temperate phages may recombine with bacterial genes. Evidence for this comes from the fact that some

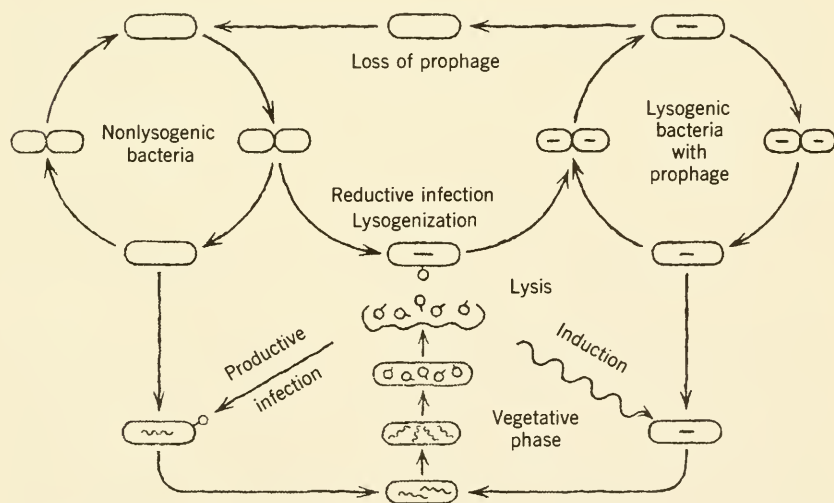


FIGURE 5.5. The life cycle of a temperate phage (after Lwoff, 1953, *Bact. Rev.*, 17:269).

semi-temperate phages, when plated on a certain bacterial host, yield mutant types which, more frequently than chance will allow, occur together in a single cell as though they had both arisen in a single event. As the progeny phages are repeatedly plated on the same bacterial strain, they become stable. But immediately upon plating on another strain mutants appear once again. It is as though the phages, by recombination with the bacterial host, picked up new genes from it. After repeated "inbreeding," the phages became isogenic with that part of the host with which they shared genetic homology. But immediately upon "outcrossing," new genes could be assimilated once again. Genetic homology with the host may be the reason for the strong resistance of temperate phages to irreversible damage by ultraviolet light. Whether there is really an exchange with the host chromosome or with some cryptic prophages on that chromosome is a problem for the future. But there may be less difference between phage and bacterial genes than was previously thought.

For example, some genes located in the prophage DNA influence traits of the host bacterium, including colony morphology and the presence of particular antigens or enzymes. This phenomenon is called *lysogenic conversion*. For example, all toxigenic strains of the diphtheria bacillus carry a specific prophage; when it is lost by cultivation in anti-phage serum, a strain is selected which is not toxigenic. When this strain is lysogenized again, it once more becomes toxigenic. Lysogenization with a different strain of phage is without effect. However, the genes of the two phages may be exchanged in a cross during vegetative reproduction, and the second phage may receive the gene conferring the capacity for toxin production on the host bacterium. These and other temperate phage genes may be mapped and are found to exist in a linear order. One group of these genes is concerned with the ability of the phage DNA to reduce to prophage. Phages bearing one of these genes in mutant form behave as virulent and invariably lyse the host unless present with another phage also mutant, but at another locus. Such "heterozygous" pairs complement one another and allow reduction of the virulent phage to the prophage condition.

Mutations also occur which make the prophage defective, that is, able to infect the host but not to multiply or to give rise to vegetative phages which lyse the host cell. The cryptic existence of defective phage is demonstrated first by the retention of the immunity against related phages that characterizes the presence of prophages. Immunity is the character a prophage confers upon its host whereby, even though a related phage may be adsorbed and inject its DNA, that DNA cannot develop into vegetative phage and rarely into prophage; it simply does

not multiply and is diluted out by division. Second, the occurrence of mutations from the defective state back to normal identifies defective phages. These rare changes allow the release of some free normal phages in a culture carrying a defective prophage, but that culture, although lysed upon exposure to inducing agents, does not produce phages in the process. Therefore we can see how mutation to the defective state suppresses the viral aspect but not the other genetic functions of the prophage. The sequence — virus to prophage to defective — is a change from infectious heredity to stable heredity. The reverse mutation from defective phage to prophage causes the special character of the infected cell to become transmissible via the virus produced (Figure 5.6).

TRANSDUCTION OF BACTERIAL GENES VIA BACTERIOPHAGE

By phage infection, then, genes determining bacterial characters may be transferred from one host to another. Lysogenic conversion is nonetheless quite different from *transduction* via temperate phages. In transduction the phage is simply a vehicle for the transfer of a part of the genotype of the donor bacterium to a recipient bacterium. When streptomycin-sensitive bacteria, for example, are infected with phages that have been grown on streptomycin-resistant hosts, a fraction (ca. 10^{-6}), of the cells that survive infection become streptomycin-resistant and can thenceforth act as donors of that character. During their multiplication some temperate phages incorporate within their coats not only the DNA of their own genotypes but also some DNA from their bacterial host. This host DNA is injected into the recipient and has a small

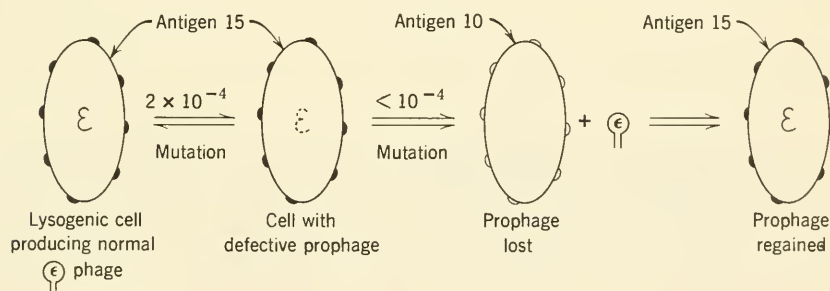


FIGURE 5.6. Mutation to defectiveness and loss of prophage in a *Salmonella* lysogenic for phage ϵ which causes the formation of antigen 15 instead of antigen 10 produced in its absence (after Uetake, in: Luria, 1958, *Ann. N. Y. Acad. Sci.*, 71:1085).

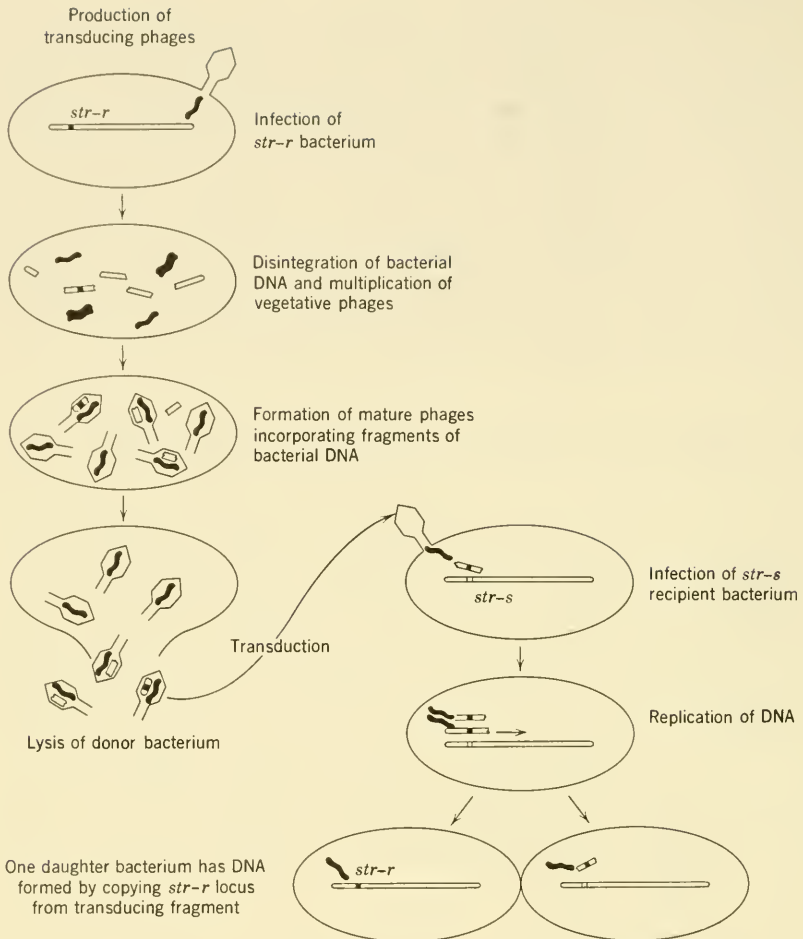


FIGURE 5.7. A diagram of the transduction process showing some of the steps that may be involved.

chance of becoming incorporated in the host's genotype. In Figure 5.7 the host DNA is shown as a fragment separately picked up by the phage coat; as we shall see, at least in some cases, part of the DNA of the transducing phage has been replaced by host DNA through a process of recombination.

In the case of lysogenic conversion, the genotype of the bacterial donor of the phage makes no difference; the genotype of the prophage alone determines conversion. On the other hand, in transduction, the donor's genotype is critical. For example, phages grown on a trypto-

phaneless strain will not transduce tryptophaneless bacteria of the same strain which are, of course, mutant at the same site. To succeed, the phages must be grown on tryptophane-synthesizing bacteria, or at least on bacteria that have the wild-type allele of the locus that is mutant in the recipient strain. A suspension of sensitive bacteria infected by transducing phages may simultaneously be transduced and undergo lysogenic conversion. But the two processes may also occur independently in the same suspension; some bacteria may be lysogenized and not transduced—they are converted. Others may be transduced and not converted. In this case the infection is initiated and the contents of the phage coat are injected but, for some reason, the phage DNA aborts and neither enters the productive cycle nor becomes reduced to prophage. Only the bacterial DNA is incorporated and assumes a function.

Sometimes a phage may even carry from cell to cell the prophage of

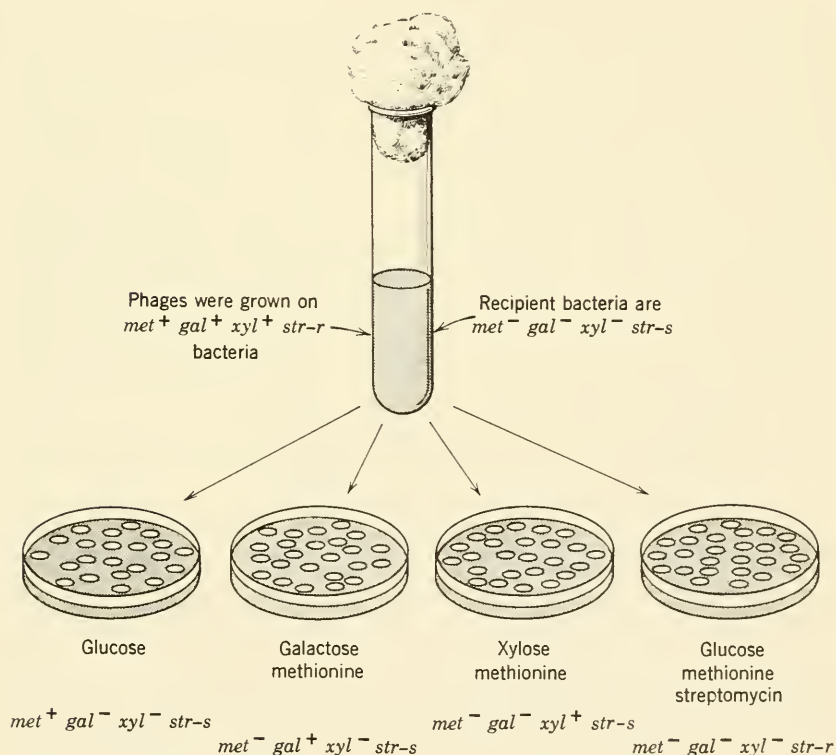


FIGURE 5.8. Transduction of one character at a time in *Salmonella typhimurium* (after Zinder and Lederberg, 1952, *J. Bact.*, 65:679).

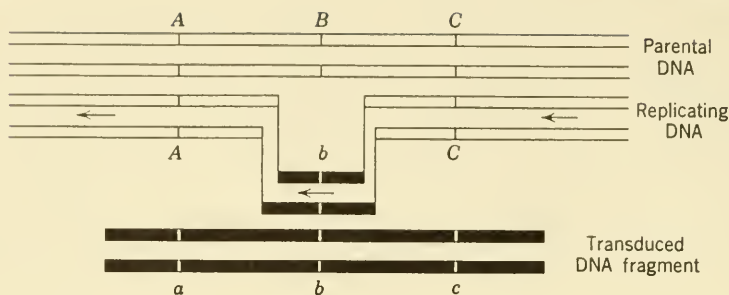


FIGURE 5.9. A possible copy-choice mechanism for the replacement of recipient by donor genes after transduction.

another type. Thus the coat of a transducing phage may enclose several kinds of DNA and, in fact, transducing phages can be separated from others by centrifugation on account of their different density. But the nonphage DNA never includes as much as the whole bacterial genotype, only fragments of it. We might have anticipated this because, during phage development, the host DNA degrades, some of it even to the level of purine and pyrimidine bases. We have further assurance from the pattern of transduction itself.

When temperate phages are grown on a strain of the bacterium, *Salmonella typhimurium*, that is able to synthesize methionine, utilize the sugars galactose and xylose, and resist streptomycin ($met^+ gal^+ xyl^+ str-r$), and are then allowed to infect a strain which has the opposite characters ($met^- gal^- xyl^- str-s$), some of the latter survive and are transduced. But such cells are transduced for only one character (Figure 5.8). When, for example, selection is made on agar with streptomycin, $str-r$ transductants are found, but they are still $met^- gal^- xyl^-$, and so on. It was the same with transformation in pneumococcus; penicillin-resistance and streptomycin-resistance were conferred independently upon the transformed cells. Both in transformation and in transduction most genes are transferred independently; the pieces transferred are parts, and not the whole, of the genotype. But if two genes are closely linked, they may be transduced together with a frequency that is a function of the closeness of their linkage. On this principle bacterial genes can be mapped by transduction, and their order is found to be that observed by usual crossing techniques. Another method of mapping utilizes only the relative frequencies with which various mutants will transduce one another (Chapter 6).

It may be supposed that the transduced DNA pairs with the host DNA

and acts as a template for the replication that next occurs. Clearly, such a copy-choice mechanism will result in the substitution of one gene for another in the newly formed DNA. If two mutants differ in the site that has mutated, one can transduce the other; if the mutations are at the same site, a wild type cannot result from transduction (Figure 5.9). Thus a series of *gal*⁻ mutants can be arranged into groups according to whether one can transduce the other. When transduction does not occur, it is likely that the two mutants have lesions at the same or closely linked sites; when transduction does occur, we can conclude with assurance that the sites are different. There seems to be some preference in the sequence of nucleotides which will be copied, for it appears that the wild-type allele may more easily replace its opposite in transduction than vice versa. This model accounts for the incorporation of the information carried in the transduced DNA into the replicating genetic material of the recipient, and so it should, for transduced material usually multiplies in synchrony with the rest of the genotype.

Sometimes, however, the transduced bit of DNA takes up residence in the new host cell but does not multiply at all. It controls the phenotype of the cell but, upon division, is passed to one or the other daughter. Such a situation is referred to as *abortive transduction*, and the cells that contain the transduced DNA in series are called a *line* (in contrast to a clone, which expands in number). A striking example of abortive transduction involves flagella formation, and hence motility, in *Salmonella*.

When a flagellaless culture is treated with phage from a motile one, some of its members are transduced to motility. Stable transductants divide and swim away from the parent colony, forming extensions from it (Figure 5.10). Abortive transductants also swim away but, upon divi-

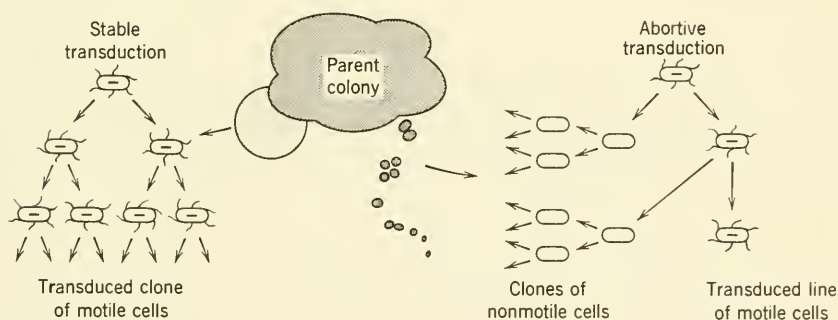


FIGURE 5.10. Two types of transduction in *Salmonella*, stable and abortive. Non-motile bacteria mixed with phages from a motile strain form a parent colony from which transductants emerge (after Stocker, 1956, *J. Gen. Microbiol.*, 15:575).

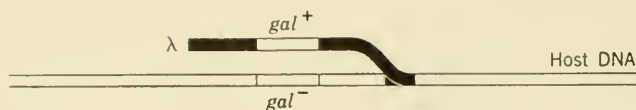


FIGURE 5.11. A possible structure of the *gal* region of the chromosome in an *E. coli* heterogenote.

sion, each leaves behind a nonmotile cell which forms a colony at that site. The motile sister swims away and divides again, repeating the process. Thus it leaves a trail of separate, small colonies in its wake. Trail formation can actually be observed under the microscope. When the abortively transduced DNA carries into an auxotrophic host a gene causing synthesis of the growth factor, a line is formed which increases arithmetically in number, instead of exponentially. Such abortive transductions give rise to minute colonies.

Another kind of association sometimes occurs in which the transduced piece of DNA may duplicate in synchrony with the host DNA, but does not replace the homologous gene of the host. The bacterium transduced in this way is a heterozygote for the transduced region; strictly speaking, it is a *heterogenote*, because only a small bit of genotype is represented twice. Heterogenotes are formed by a temperate phage called lambda (λ), which is found in *E. coli*. This phage can locate itself at only one site on the bacterial chromosome, at a place very close to the *gal* locus. Although other phages may transduce many *E. coli* genes, λ can transduce only *gal*, to which its prophage is linked. As a matter of fact, λ can only transduce when it is formed directly from prophages. When λ is grown on a sensitive cell that is *gal*⁺ but without having taken up a lysogenic association with that gene, it cannot transduce it. It would appear that when the prophage is released from the chromosome upon induction, it may take a little more than itself along. Phages that have picked up the neighboring *gal* locus reduce to a defective state, so that once they have established themselves as prophages in the new hosts they do not form infectious particles. Nor does the complex replace the equivalent loci in the host; both the transduced *gal* locus and that in the host are present together, forming the heterogenote (Figure 5.11). At a rate of ca. 10^{-3} per cell per generation these heterogenotic associations may break down, and haploid bacteria segregate with the original *gal* character, with the transduced character, or with recombinations of the several *gal* markers that may have been involved in the original transduction. Very rarely bacteria segregate which become homozygous at the *gal* locus. The rare phage produced spontaneously by heterogenotes,

presumably by mutation from the defective state, can transduce the *gal* marker with an efficiency approaching 100 per cent. This is probably due to the fact that the defective prophage and the attached *gal* locus form a tightly integrated unit loosely attached to the genotype of the heterogenote.

During the multiplication that follows the usual invasion by an exogenous phage, there is only a small chance for any locus from the disintegrating bacterial chromosome to become included within a phage and to be transduced. And then again, there is only a small chance that, once carried over, it will be incorporated in the genotype.

TRANSFORMATION BY DNA

There is really little difference between transduction and transformation, other than the way the DNA fragment is transferred, by a phage or by an experimenter. In both processes, exogenous DNA is incorporated into the genome of the receptor cell. It seems likely that the mechanism of incorporation is similar in the two systems, perhaps like that presented in Figure 5.9, but there is little experimental evidence on this question as yet. Of course, in transduction the bit of DNA in the phage coat is protected from the destructive action of the enzyme, deoxyribonuclease (DNA-ase), and the extracted fragment in transformation must find its own way into the cell. The latter may be achieved through special receptor sites on the host cell surface. We suppose this as a result of experiments in which a nontransforming DNA is used as an inhibitor. If the competition is for adsorption, then the nontransforming DNA will depress equally both the uptake of transforming DNA and the number

TABLE 5.3

Inhibition by Nonradioactive, Nontransforming DNA of the Uptake of P^{32} -Labeled Transforming DNA and of the Number of Transformants

(From Schaeffer, 1958, in *Biological Replication of Macromolecules*.

New York: Academic Press, p. 60)

Concentration of Inhibitory DNA	Percentage Inhibition of	
	Retention, by DNA-ase Treated Bacteria, of P^{32} DNA	Transformation
0	0	0
3	44	42
10	73	71
30	87	86

of transformants, whereas the latter only will be lowered if competition occurs inside the bacterium. As Table 5.3 shows, both are inhibited equally. Furthermore, when cells are exposed to a highly polymerized extract of DNA marked with P^{32} , the tracer is found in the cell and can be released again if the cell is lysed artificially soon after penetration. But when such P^{32} DNA is broken into smaller pieces, it does not enter. This sets a minimum size for the transforming particle, but as was the case with transduction, there is in practice also a maximum size, for most bacterial genes are transformed independently (see Table 1.3).

However, when genes are closely linked, they may be transformed together as we saw was the case with streptomycin resistance and mannitol fermentation in pneumococcus (Table 1.4). We suppose that the excess double transformants are a function of structural linkage in the DNA, and that incorporation is actually a recombination and follows some copy-choice mechanism, as in the case of transduction.

The recombinations being discussed are, in the final analysis, events occurring between DNA molecules. Although our concepts of the mechanism of these events may need modification, it is clear that new types of DNA are the result. Transformation experiments give the sharpest evidence for this thesis. A good case is that of transformation between strains of pneumococcus with different amounts of capsular antigen. Type SIII-N is a smooth, wild type, and possesses a fully developed capsule; DNA extracted from it and applied to unencapsulated, rough (R) cells yields some transformants, and all are SIII-N like the donor of the DNA. In addition, there are intermediate smooth types such as SIII-2, SIII-1, SIII-1a, SIII-1b, and SIII-1c, which have smaller

Some Interactions Between Pneumococcus Cells of Intermediate Capsule Content and Their DNA's

(From Ephrussi-Taylor, 1951, *Exp. Cell. Res.*, 11:589)

Treated Strain	Source of DNA	Results
SIII-1	SIII-1a	SIII-1a + SIII-2a
SIII-1a	SIII-1	SIII-1 + SIII-2a
SIII-1	SIII-1b	SIII-1b + SIII-2a
SIII-1	SIII-1c	SIII-1c + SIII-N
SIII-1c	SIII-1	SIII-1 + SIII-N
SIII-1	SIII-2	SIII-2 + SIII-N
SIII-1a	SIII-1c	SIII-1c + SIII-N
SIII-1c	SIII-1a	SIII-1a + SIII-N
SIII-1a	SIII-2	SIII-2 + SIII-N
SIII-1b	SIII-2	SIII-2 + SIII-N
SIII-1c	SIII-2	SIII-2 + SIII-N

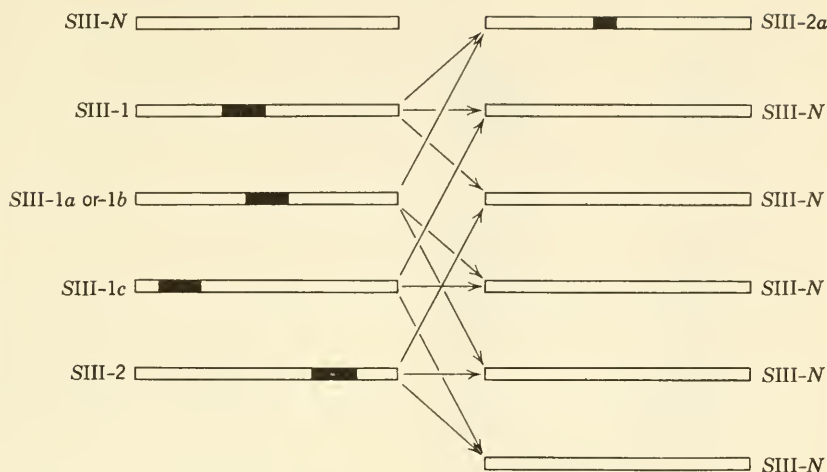


FIGURE 5.12. A scheme for the structure of the DNA in the strains listed in Table 5.4, which will explain the results obtained. Only a few interactions are indicated as an example of how the model works.

amounts of capsule; each one yields DNA which will transform *R* cells only to its own type. But when these DNA's are used to transform one or another of the intermediate types, results of the sort shown in Table 5.4 are obtained. At first glance these seem indeed to be complex results, but there is a very simple explanation. You will note that most transformations are to the same type as that of the DNA employed; these are called autogenic. But some are to the wild-type condition and are called allogenic. The wild type was not involved in the experiment, however, either as the treated cell or as the source of the DNA, and mutations to that condition are not frequent enough to account for the allogenic transformations. This suggests that recombination is at work. If we assume that the different types have DNA's of the structure shown in Figure 5.12, allogenic transformation can be explained on the basis of a copy-choice mechanism of DNA duplication. The validity of this model is yet to be attested, but it is clearly established that all transformants contain DNA characteristic of their phenotypes. Therefore new types of DNA are produced in transformation by some mechanism of recombination.

These new SIII-N types do not subsequently break down into their component parts in pneumococcus but in another bacterium, *Hemophilus influenzae*, where the situation is formally the same, they do so. DNA from an *Hemophilus* strain with an antigenic capsule of type *a* will trans-

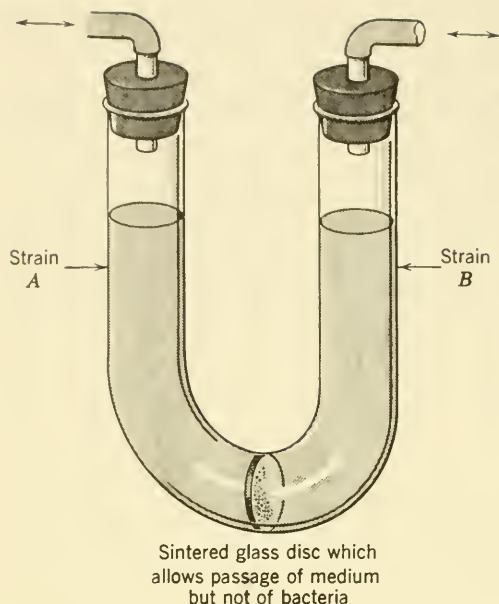


FIGURE 5.13. The U-tube used to show that cell contact is required for bacterial recombination.

form cells with a capsule of type *b*, sometimes yielding *ab* transformants with both antigens in their capsules. The association of the two DNA's is usually retained so that the DNA extracted from the doubly encapsulated types sometimes gives linked transformations; but the coupled marker genes may also separate so that cells are transformed for one or the other.

MATING IN BACTERIA

Some bacteria, such as *E. coli*, may exchange their genes by a mechanism which involves cell contact. Many different lines of investigation show that this mechanism does not involve either virus or free nucleic acid molecules; no filterable agent is necessary. For example, a U-tube is used with a bacterial filter at the base separating the two arms (Figure 5.13). In each of these arms is a suspension of *E. coli* of a different genotype. By alternately applying pressure to the air above the suspension in each arm, the liquid is forced through the filter in both directions and mixed. But the bacteria cannot pass through, and each type remains separated. Under these conditions, recombination is never observed. It only occurs when the two strains are in the same suspension

where they can come in contact. Of course, it could be suggested that a filterable substance is still the agent of gene exchange but that it is adsorbed on the filter, or has a very short half-life. The assemblage of facts to be presented precludes this interpretation.

Actually, the bacteria can be observed to join with one another in pairs. In this behavior there is a system of polarity; not every strain of bacteria will mate with every other strain. Those that are referred to as F^- will not mate with each other, but they will mate with certain other strains called F^+ and Hfr . The Hfr (high frequency of recombination) strain is by far the most active. When Hfr bacteria are genetically marked by factors for motility and cell shape, they can be distinguished microscopically in the same suspension from F^- bacteria possessing the opposite characters. In such mixed cultures it is observed that members of the two strains quickly pair, and join together across a narrow cytoplasmic bridge (Figure 5.14). This attachment is temporary, lasting about an hour. The paired members then separate and each exconjugant divides to form a clone. But only the F^- clone gives recombinant progeny; the Hfr parent continues to produce its original type exclusively. This suggests first that there has been a one-way transfer of genetic material from Hfr to F^- and, second, that since the Hfr parent survives, the transfer does not include all of its genes. But we know that the cells of *E. coli* usually have more than one set of genes, in the form of several haploid DNA-containing bodies, so it might be asked whether a whole or an incomplete set is transferred across the cytoplasmic bridge.

Lederberg, in his original work on this subject, found that certain genes of the Hfr strain were very frequently absent from the recombinants; in the strain he was using these included the loci for streptomycin resistance and maltose utilization. Evidence on this point came from his studies on nondisjunctional strains, which were produced as progeny of crosses in which a gene called *het* is present. In this case,

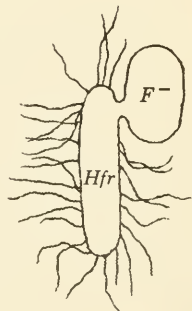


FIGURE 5.14. A conjugating pair of *E. coli* formed between morphologically different members of an Hfr and an F^- strain (after Lederberg, 1956, *J. Bact.*, 71:497).

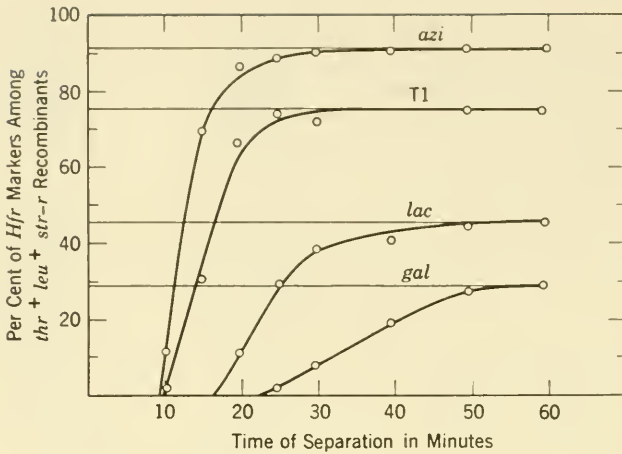


FIGURE 5.15. The time course of transfer of genes from an *Hfr* to an *F*⁻ strain as determined by the method of interrupted mating. A mixture of *Hfr thr*⁺ *leu*⁺ *azi*-*S* *T1*-*s* *lac*₁⁺ *gal*_b⁺ *str*-*s* and *F*⁻ *thr*⁻ *leu*⁻ *azi*-*r* *T1*-*r* *lac*₁⁻ *gal*_b⁻ *str*-*r* bacteria is made, agitated in a Waring Blendor and plated on minimal medium plus streptomycin so that only the *thr*⁺ *leu*⁺ *str*-*r* recombinants are recovered (after Wollman and Jacob, 1958, *Ann. Inst. Pasteur*, 95:641).

in the clone formed from mated bacteria, many of the cells remain heterozygous for a number of genes. Even after single cell isolation the resulting clone consists of a mixture of cells heterozygous for some genes, and two kinds of haploid cells containing one or the other of the genes of a heterozygous pair. For example, if a cell heterozygous for *lac*⁺ and *lac*⁻ is isolated, it produces some progeny still heterozygous and others in which the *lac*⁻ has segregated from the *lac*⁺. Among the segregants all combinations of parental genes are found, indicating that the heterozygote is not just a *heterokaryon* that carries the two different parental nuclei in their original form with a chance that they will separate at division.

The heterozygote *lac*⁻/*lac*⁺ has a *lac*⁺ phenotype because that gene is dominant. But sometimes, when the parents in the cross are *lac*⁻ and *lac*⁺, the unreduced progeny are *lac*⁻ and produce only *lac*⁻ offspring, although they continue to segregate the two alleles of other markers for which they are heterozygous. It can be shown in the following way that such strains are actually homozygous for *lac*⁻. From the strain in question a *lac*⁺ mutant is selected. Since mutations occur independently, if the strain were of the genotype *lac*⁻/*lac*⁻, it should have become *lac*⁻/*lac*⁺ by mutation, and as a consequence should now segregate both *lac*⁻

and *lac*⁺. This is found to be the case. But it is otherwise with the *mal* locus. The heterozygotes from a *mal*⁺ × *mal*[−] cross are almost invariably either *mal*⁺ or *mal*[−], and they produce only the same type of offspring. If a mutant to the *mal*⁺ condition is selected from the heterozygote that is *mal*[−], it produces only *mal*⁺ offspring, unlike the mutated *lac*[−] homozygote which after mutation segregated both types. The heterozygote that was *mal*[−] must, therefore, have had that gene represented only once; heterozygous for other genes, it was *hemizygous* at the *mal*[−] locus. Such strains could have become hemizygous after a complete homozygote was formed, or, because the *mal* gene in the hemizygote is usually that of the *F*[−] parent, such a condition may never have existed and the genetic contribution from the *Hfr* parent may have been only partial, giving rise to a merozygote.

Although genetic material may sometimes be lost from the zygote, Wollman and Jacob, at the Pasteur Institute in France, showed that the absence of particular genes among the recombinant progeny of a cross results from incomplete transfer of genetic material. Their method consisted of separating the members of pairs at different times during the conjugation process by agitating them vigorously in a Waring Blendor. It was found that the number of genes transferred from the *Hfr* was directly proportional to the time elapsing before the cells were separated. Remarkably, one gene was always transferred first, and the others followed in order (Figure 5.15) as though the genetic material were arranged in linear sequence and passed lengthwise across the cytoplasmic bridge. When the pairs are agitated, the bridge is broken, leaving in the *Hfr* cell whatever genes have not yet been transferred. No matter how long the cells are allowed to pair, some genes rarely enter the *F*[−] cell. Apparently spontaneous breakage usually prevents distant genes from entering.

TABLE 5.5

Genetic Map of the Chromosomal Segment Injected with High Frequency in One Strain of *Hfr*

(From Jacob and Wollman, 1958, *The Biological Replication of Macromolecules*. New York: Academic Press, p. 75)

	<i>thr</i>	<i>leu</i>	<i>azi</i>	<i>T</i> ₁	<i>lac</i> ₁	<i>gal</i>	
A	—	—	90	70	40	25	15
B	8	8½	9	11	18	25	26

A is the per cent of *thr*⁺ *leu*⁺ *str*-r recombinants which have inherited the different *Hfr* alleles.

B is the time in minutes at which individual *Hfr* characters start penetrating the *F*[−] recipient.

The method of interrupted mating allows easy mapping of that part of the genetic material which usually gets into the F^- cells (Table 5.5). The order of genes in such maps corresponds to that found by the more classical techniques of recombination analysis, which depend upon the assumption that recombination occurs in a zygote with two complete sets of genes. The procedure is as follows. Two mutant strains differing in a number of genes are mixed together. The recombinants are rare and must be selected for by a suitable choice of parental strains. For example, one might be threonineless-leucineless ($thr^- leu^-$) and the other, methionineless-biotinless ($met^- bio^-$). Such strains by themselves will not form colonies on agar devoid of the required substances because double back mutations of the $thr^- leu^-$ or of the $met^- bio^-$ in the same cell are too rare to be observed. But when the two strains are mixed and then plated on minimal agar, some prototrophs ($met^+ bio^+ thr^+ leu^+$) with genes from each parent are recovered (Figure 5.16). These results do not provide linkage data because only one recombinant type is selected, it is not known how many effective matings occurred, and no parental frequencies are observed.

However, it is possible to establish linkage relations by crossing strains which are double auxotrophs and also carry other markers which are not deliberately selected for or against. The unselected markers should be found in the prototrophs with a frequency that is a function of their linkage to the selected markers. If a gene is very closely linked to a selected marker, it will be found among the offspring with a high frequency. In a reciprocal cross it will, of course, be represented less frequently. For example, in a cross of $met^- bio^- thr^+ leu^+ lac^+ T1-r \times met^+ bio^+ thr^- leu^- lac^- T1-s$, where $T1$ is a locus determining resistance to phage $T1$, the prototrophs can be selected for and the frequency with which the lac and $T1$ markers appear among them can be determined. Typical results are shown in Table 5.6. It will be seen that most of the offspring were lac^- , showing a linkage to the $met bio$ loci. $T1-r$ types are also in preponderance, indicating a linkage of that locus with $thr leu$, which in this method must be considered a unit. The frequency of recombination between the lac and $T1$ loci indicates free exchange between them. But there is a marked deficiency of one class of recombinants, $lac^+ T1-s$, compared to the reciprocal, $lac^- T1-r$. This can be explained by assuming that the lac and $T1$ loci are between those for $met bio$ and $thr leu$ as the diagram in Table 5.6 shows. It can also be seen that the parental coupling determines the frequency of a given gene among the progeny because of the selection for one and against the other marker to which the gene is linked. Nonetheless, the exchanges in a given region of the linkage group are about the same in each cross, and a map can be con-

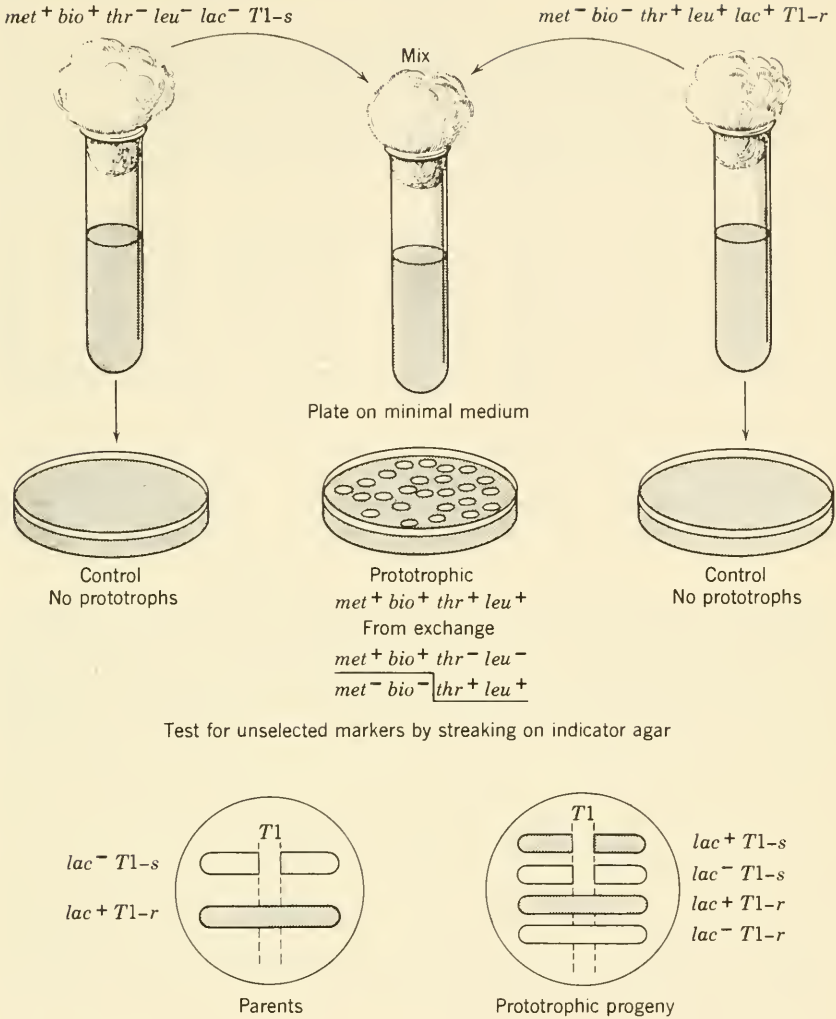


FIGURE 5.16. The result of plating a mixture of $thr^- leu^-$ and met^- auxotrophic *E. coli* on agar devoid of the required growth factors (after Lederberg, 1947, *Genetics*, 32:505).

structed in which the distances are roughly additive. The frequency of triple exchanges expected ($0.26 \times 0.47 \times 0.33 = 0.04$) is almost the same as that observed (0.02). Even numbers of exchanges within this region would not, of course, be selected by the method used.

A similar order of genes is observed by a completely different tech-

TABLE 5.6

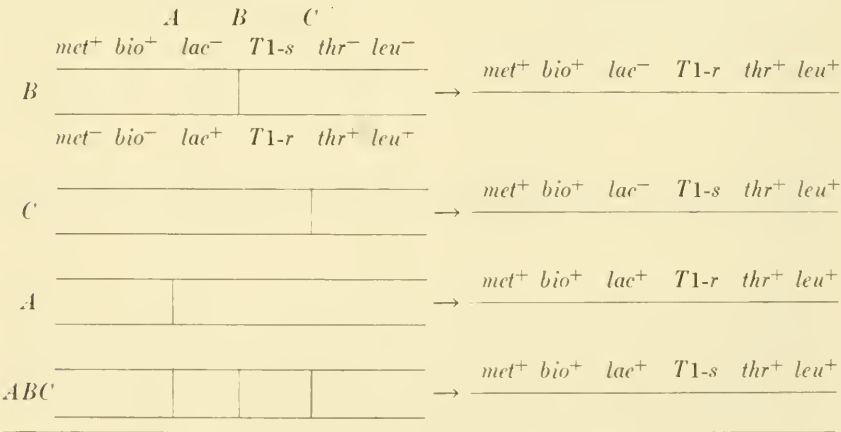
The Genotypes of Offspring from a Cross of
met⁺ bio⁺ thr⁻ leu⁻ × met⁻ bio⁻ thr⁺ leu⁺ E. coli with
Different Couplings of the Loci for *lac* and *T1*

(From Lederberg et al., 1951, *C. S. H. Symp. Quant. Biol.*, 16:413)

Parents				Total Number	Prototrophs <i>met</i> ⁺ <i>bio</i> ⁺ <i>thr</i> ⁺ <i>leu</i> ⁺ Per Cent of Different Genotypes			
<i>met</i> ⁺ <i>thr</i> ⁻	<i>bio</i> ⁺ <i>leu</i> ⁻	<i>met</i> ⁻ <i>thr</i> ⁺	<i>bio</i> ⁻ <i>leu</i> ⁺		<i>lac</i> ⁻	<i>T1-r</i>	<i>lac</i> ⁺	<i>T1-s</i>
<i>lac</i>	<i>T1</i>	<i>lac</i>	<i>T1</i>		<i>lac</i> ⁻	<i>T1-r</i>	<i>lac</i> ⁺	<i>T1-r</i>
-	<i>s</i>	+	<i>r</i>	2013	42.7 <i>B</i>	23.2 <i>C</i>	32.5 <i>A</i>	1.6 <i>ABC</i>
-	<i>r</i>	+	<i>s</i>	696	34.6 <i>C</i>	42.5 <i>B</i>	26.1 <i>ABC</i>	20.3 <i>A</i>
+	<i>s</i>	-	<i>r</i>	518	25.1 <i>A</i>	2.5 <i>ABC</i>	47.7 <i>B</i>	24.7 <i>C</i>
				26	47	33		
<i>met bio</i>				<i>lac</i>	<i>T1</i>	<i>thr leu</i>		
<i>A</i>				<i>B</i>	<i>C</i>			

The letters indicate the regions of the linkage group in which an exchange must occur for a recombinant of the type indicated to be produced. Another exchange must occur, of course, outside of this region to incorporate the genes in the fragment from the *Hfr* into the recombinant DNA. The distances on the linkage group are the averages of the frequency of exchange occurring in the different regions.

Example of the Exchanges that Occur in the
First Cross Above to Give the Results Tabulated



nique. When *Hfr* are labeled with P^{32} , it is found that this tracer will pass with the genetic material into the F^- mate, and in a reciprocal experiment it can be shown not to pass in the other direction. Now if the *Hfr* DNA is labeled with P^{32} , and if this element is allowed to disintegrate to different extents by storage in the cold before the cross is made, then the likelihood that a gene will be transferred is proportional to its distance from the anterior end of the chromosome. This method is, in principle, like the interrupted mating technique. The longer P^{32} is allowed to disintegrate, the more likely is the occurrence of a chromosome break that will prevent the transfer of genes distal to it. Genes farther from the front end of the chromosome are thus more likely to be separated and left behind than more anterior genes. The relative rates of loss of the individual marker genes is similar to the relative times of entry of the same markers, as Table 5.7 shows. The coincidence of results suggests that the P^{32} is uniformly distributed along the chromosome and that the chromosome enters the F^- at a constant rate of about 10^3 nucleotide pairs per second. There seems little doubt about the linear order of genes in the bacterial linkage group.

There is considerable doubt, however, about the nature of the bacterial chromosome, and little information about the mechanism of exchange itself. Bacterial cells can be shown to possess microscopically visible "nuclei," but these have not been shown to divide by mitosis. They have, on the other hand, some of the basic properties of chromosomes in higher forms. They are Feulgen-positive because they contain DNA, and they are duplex, as shown by the experiments of Meselson and Stahl (Chapter 7). Furthermore, Witkin has shown that they are probably the sites occupied by some bacterial genes. She found that *lac*⁻ mutants induced by ultraviolet light usually appeared as whole *lac*⁻ colonies, half colonies, or quarter sectors according to whether the cells irradiated were preponderantly uninucleate, binucleate, or quadri-nucleate. Whether the structure of these nuclei involves other constitu-

TABLE 5.7
Comparison between the Relative Rates of Entry of Genes on the
Hfr Chromosome with the Relative Rates of Loss of the Same Genes after
 P^{32} Disintegration

(From Jacob and Wollman, 1958, in *The Biological Replication of Macromolecules*. New York: Academic Press, p. 75)

	<i>thr leu</i>	<i>azi</i>	<i>T1</i>	<i>lac</i> ₁	<i>gal</i> _b
Rate of loss	0.35	0.39	0.43	0.67	1.00
Rate of entry	0.34	0.36	0.44	0.72	1.00

ents than DNA, and more complexity than a supercoiling of a long, double helix of DNA, and whether their mode of division is more complex than that of DNA itself are questions to be answered in the future.

We can ask now, however, whether the bacterial zygote, which is clearly partial, undergoes something akin to meiosis in the production of its haploid progeny among which the recombinants are found. It was already noted that among the progeny of the persistent heterozygote, homozygotes were obtained. This means that the quasi-diploid heterozygous products of the zygote do not always resemble it in genotype. A genetic event has intervened which is capable of making some loci homozygous. The same was sometimes true in the transduction heterozygote. In Chapter 12 such a mechanism will be described as it operates in the chromosomes of fungi, yeast, and *Drosophila*. It involves crossing over during mitosis; but it is also possible to account for the bacterial results in terms of a copy-choice mechanism during the replication of DNA. The isolation of the single-cell products of conjugating bacteria and of persistent heterozygotes gives results that are irregular enough to suggest the latter possibility. Sister cells are usually not reciprocal recombinants; in fact, the deviation from equality of reciprocal recombinants in a single clone may be as much as 15:1. Apparently the fragment of DNA from the *Hfr* persists and repeatedly acts as a template in recombination until it is lost, perhaps in a nonviable daughter cell.

In conformity with the copy-choice hypothesis of recombination is the fact that there seems to be a polarity of incorporation of the newly arrived *Hfr* genetic material into the recombinant chromosome. Although the *thr*⁺ *leu*⁺ markers of a particular *Hfr* strain may enter nearly 100 per cent of the zygotes, only 20 per cent form *thr*⁺ *leu*⁺ recombinants. Furthermore, when some 60 per cent of the zygotes receive the *gal*⁺ marker, only 5 per cent form recombinants containing that locus. The disparity between the efficiency ratios of 1:5 and 1:12 suggests some zipper action. On the partial replica hypothesis, in order to produce prototrophs one switch must occur in front of the *thr leu* loci and another distal to them; the closer a marker is to the distal end of the introduced fragment of DNA, the less likely it is to be incorporated in this partial replica. Again, ultraviolet light increases the chance of recombination and, therefore, reduces the frequency of unselected markers from the *Hfr* among the progeny. As was the case with irradiated phages, lesions produced randomly in the irradiated genetic material interfered with the process of replication and increased the frequency with which the replicating element shifted from one chromosome to another.

Similarly, when radioactive *Hfr* are mated with nonradioactive *F*⁻ and

P^{32} decay is allowed to occur in the zygotes just after mating, the types of recombinants can be measured as a function of that decay, and its effect upon integration will be determined independently of any effect on transfer. The linkage of genes in the *Hfr* parent is decreased with time and in proportion to their distance from the *thr leu* markers at the head end of the chromosome, suggesting that the P^{32} disintegration destroys the integrity of genetic material and that the sensitivity of any segment is proportional to its length. This combination of physical with genetic studies has not yet clarified the question of how *Hfr* genes are incorporated into the F^- genetic material. The polarity so evident in the experiments just reported could be no more than a pairing phenomenon. Although the results do not demonstrate it, they are compatible with a copy-choice mechanism of the sort shown in Figure 5.9. The critical experiments are yet to be done.

Analysis of the mating system of *E. coli* has led to a much clearer understanding of the organization of the bacterial chromosome than has recombination analysis by itself. F^+ bacteria possess a substance (called *F*) which is contagious, transforming F^- into F^+ upon contact. All the progeny of an $F^+ \times F^-$ cross are F^+ by infection. The offspring of an *Hfr* $\times$ F^- cross are almost invariably F^- ; there is no contagious substance in *Hfr* cells. F^+ cultures consist of a mixture of cells that are phenotypically F^+ and some that temporarily behave as F^- ; similarly, *Hfr* cultures are mixtures of cells that behave as *Hfr* and as F^- . Thus intratype crosses produce recombinants. Beyond that, F^+ cells can lose their *F* substance irreversibly and become F^- by growth in the presence of cobalt or acridine dyes. F^+ cells may also mutate to *Hfr*; in fact, it seems that the only cells which are actually able to mate in an F^+ population are the *Hfr* mutants. Wollman and Jacob showed this with the replica plating technique (p. 52). They imprinted the surface of a plate spread with $F^+ thr^- leu^-$ bacteria onto velvet and replicated this onto a plate of minimal agar spread with $F^- met^- bio^-$ bacteria. Only the recombinants resulting from a mating formed colonies on the second plate. When isolations were made from homologous positions on the first plate, pure *Hfr* clones were obtained. Each of these had a mutational origin and, interestingly, each independent *Hfr* had a different genetic structure, as shown by interrupted mating experiments. The same order of genes was found, but each independent *Hfr* mutant had a different gene at the anterior end of its linkage group, as shown in Table 5.8. From data such as these, it can be seen that every gene is linked to every other, as would be the case if the chromosome were not a rod but a continuous structure with no end. It can be represented as a circle, but such a structure could not transfer genes from the *Hfr* to the

TABLE 5.8

Orders of Genes in a Variety of *Hfr* Mutants(From Jacob and Wollman, 1958, in *The Biological Replication of Macromolecules*. New York: Academic Press, p. 75)

Type	← -----
<i>H</i>	<i>thr leu azi T1 lac T6 gal λ - - -</i>
1	<i>leu thr B1 met mtl xyl mal str - - -</i>
2	<i>T1 azi leu thr B1 met mtl xyl mal str - - -</i>
3	<i>T6 lac T1 azi leu thr B1 met mtl xyl mal str - - -</i>
4	<i>B1 met mtl xyl mal str λ gal - - -</i>
5	<i>met B1 thr leu azi T1 lac T6 gal - - -</i>

F^- in a linear sequence. Therefore we must suppose that in each *Hfr* the chromosome is not a circle, but rather that it is a rod.

All these facts can be resolved if we assume, as in Figure 5.17, that in the F^- cells the chromosome is a circular structure. In F^+ cells there must also be the contagious *F* factor, probably not in the linkage group because it has not been found to be linked with any other factor, and because it can be irreversibly lost. Unless the *F* factors are divided by a regular mechanism, there must be a fair number of them present because random distribution during cell division very rarely gives rise to an F^- daughter and, moreover, they can be transferred to F^- cells on contact. We may assume that the mutation from F^+ to *Hfr* represents a reduction of an *F* particle to a site on the chromosome where it induces a break. We know that an invading phage DNA molecule may reduce to a prophage on the bacterial chromosome. If the *F* factor can insert at any site, or at least at many different sites, on the chromosome, then the different classes of *Hfr* mutants can be explained. Just as in the case of prophage, the reduced *F* factor causes an immunity so that *Hfr* cells cannot be infected by *F* particles. Likewise, the unreduced particles would eventually be lost if in the presence of reduced *F* their multiplication was prevented, just as prophage prevents the multiplication of invading phage DNA. The *F* particle must be at the rear end of the chromosome because it so seldom gets into the F^- mate. This picture, speculative as it is, accounts for many facts that are not consistent with any other hypothesis yet proposed. It invokes the concept of an *episome*, a dispensable genetic unit which may multiply independently of the chromosome or as an addition to it.

The resemblance between the *F* factor and the temperate phage, both episomes, is obvious; it is strengthened by the fact that the *F* factor may

transduce bacterial genes. An *Hfr* strain which carries an *F* factor at the end of its chromosome linked to, say, a *lac*⁺ or a *pro*⁺ gene, may revert to the *F*⁺ condition in which the *F* factor becomes extrachromosomal. When this *F* factor infects *F*⁻ bacteria, many become *F*⁺ but an unusually large proportion become *Hfr*. Two interesting facts emerge: first, these *Hfr* all transfer genes in the same order and, second, they are heterogenotes for the *lac*⁺ or the *pro*⁺ factor. All results are consistent with the hypothesis that, in the original *Hfr*, the chromosomal *F* factor incorporated a neighboring gene which it carried into the next cell it infected. This incorporated material is homologous with a region of the chromosome in the new host, to which site it is attracted; this explains the "memory" of the *F* factor for the site it used to occupy. The situation is quite comparable to a λ phage which may pick up a *gal*⁺ gene from a site neighboring its point of attachment. In the case of the *F* factor, the exchange with the bacterial chromosome seems reciprocal. The cell from which the transducing *F* factor has been obtained may be cured of it by

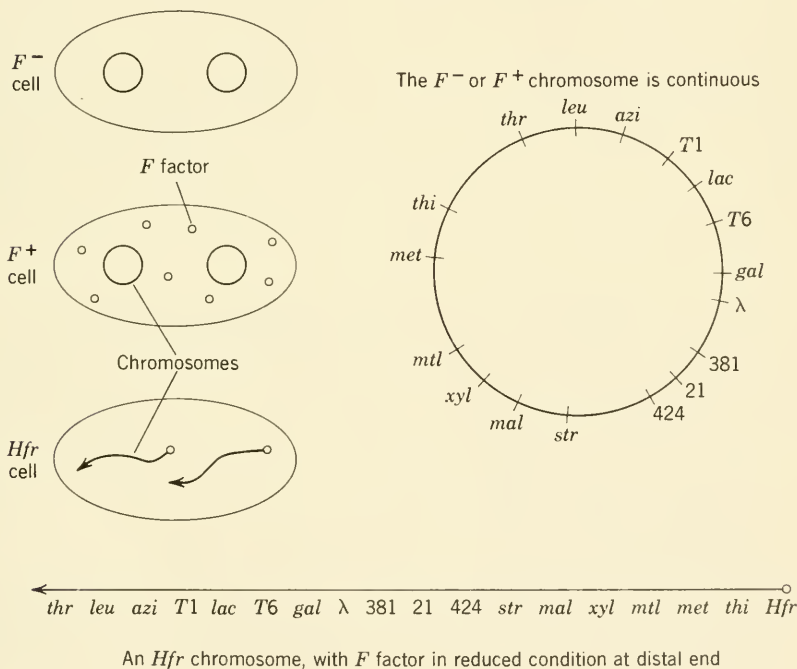


FIGURE 5.17. Speculation on the structure of the different mating types of *E. coli* (after Jacob and Wollman, 1958, in: *The Biological Replication of Macromolecules*, New York, Academic Press).

treatment with acridine orange. The F^- cells so produced, when reinfected with F particles, become, with a very high frequency, Hfr instead of F^+ . This seems to indicate that some episomal material remained in the chromosome of the F^- cell to attract the invading episome to its site. The concept of the episome and facts revealing its properties are new. Recently evidence has been gathered indicating that a factor causing bacteria to produce a toxic substance (bacteriocin) and one conferring multiple drug resistance also behave as episomes. It may be that particles such as these are of general occurrence in all cells and must be considered as a possible general mechanism of cell heredity.

The phenomenon of bacterial mating has been used to analyze many situations in *E. coli*; among the most interesting is the location of prophage. Crosses of lysogenic (λ^+) by lysogenic (λ^+) bacteria give only λ^+ progeny, just as nonlysogenic (λ^-) by nonlysogenic (λ^-) crosses give λ^- bacteria. Crosses of $Hfr\lambda^- \times F^-\lambda^+$ bacteria yield both types in a proportion that depends on the selected markers used in the cross. Thus the lysogenic and nonlysogenic factors behave as alleles at a single locus. This λ locus is closely linked to the *gal* locus. In crosses between strains carrying mutants of λ , e.g., $F^+\lambda^s \times F^-\lambda^{cl}$, no doubly lysogenic strains or sensitives are found, but each mutant appears among the recombinants linked with *gal*. The doubly lysogenic condition, which can be achieved by superinfection, is also inherited as a single locus. In contrast, a cross of a $\lambda^- \alpha^+$ strain by a $\lambda^+ \alpha^-$ strain, where alpha (α) is an unrelated prophage, does give some sensitives, as would be expected if the loci were separate. The α phage is, as a matter of fact, linked to *xyl* rather than to *gal*.

If a mating is made between an $Hfr \lambda^+$ and an $F^- \lambda^-$ strain, an unexpected result is obtained. Every time the λ prophage enters the F^- , it is induced, the F^- cell is lysed, and λ phages are released. This phenomenon is called zygotic induction. It is probably caused by the fact that when the prophage moves into the nonlysogenic F^- cell it escapes from the repression that constitutes the immunity of lysogenic bacteria. Advantage can be taken of zygotic induction to locate λ in the bacterial linkage group. Table 5.9 shows how the location of a number of linked prophages determined in an Hfr nonlysogenic $\times F^-$ lysogenic cross correlates with the frequency of zygotic induction in an Hfr lysogenic $\times F^-$ nonlysogenic cross. Furthermore, the time of entrance of the prophage can be determined as that after which agitation in the blender will not rescue the F^- from zygotic induction. This time is an inverse function of location along the chromosome.

There is also a series of noninducible prophages carried by these bacteria in the region of the chromosome where the *mal*, *xyl*, and *mtl* genes

are located. These prophages seem to be attached to the chromosome in a different way than are the inducible prophages. To demonstrate this, the fact was used that the decay of P^{32} in the genetic material of a bacterium destroys its ability to reproduce. If a prophage were in some way independent of the bacterial chromosome, P^{32} decay should kill it independently of the host. As a result, the survivors of such decay should include some nonlysogenic cells. But if the prophage were inserted in and acted as an integral part of the bacterial chromosome, a disintegration lethal for the prophage might also be lethal for the host. The failure in such experiments to recover bacteria that are free of the non-inducible prophages implies that they are inserted, at least to a considerable extent. On the other hand, the inducible prophages are easily lost by this method, suggesting that they may be inserted at a chromosomal locus at the most to a limited extent.

Another test of the continuity of the inducible prophage of λ is had in experiments in which exchanges between prophage genes are observed in a bacterial cross. Figure 5.18 shows how, if the prophages were inserted in bacterial chromosome, such exchanges would be expected to recombine outside markers, except where multiple exchanges occur in the prophage. The results are not in agreement with the insertion hypothesis. The prophage appears to be an entity, at least a part of which is an addition to the bacterial genotype. There does not seem to be in nonlysogenic bacteria an allele alternative to the whole of the

TABLE 5.9
The Location of a Variety of Inducible Prophages
in the *gal* Region of the *E. coli* Chromosome

(From Jacob and Wollman, 1957, in McElroy and Glass,
The Chemical Basis of Heredity. Baltimore: The Johns Hopkins Press, p. 468)

<i>lac</i>	<i>gal_b</i>	82	λ	434	381	21	424	466
	A	90	80	70	15	10	3	1
	B	65	55	45	30	18	12	10
	C	—	26	—	—	31	33	—

A is the per cent of *gal_b⁺ ly⁻ str-r* recombinants from crosses of *Hfr gal_b⁺ ly⁻ str-s* $\times$ *F⁻ gal_b⁻ ly⁺ str-r*.

B is the per cent of input *Hfr* whose mating results in zygotic induction in crosses of *Hfr ly⁺ str-s* $\times$ *F⁻ ly⁻ str-r*.

C is the time at which the prophage enters the *F⁻* cell when the parents are mixed at time 0.

^o*ly⁺* stands for the lysogenic and *ly⁻* for the nonlysogenic condition.

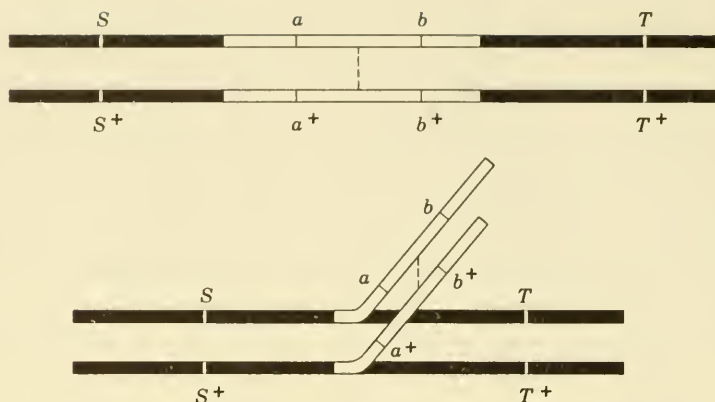


FIGURE 5.18. Diagrammatic representation of two hypotheses of prophage recombination. In the upper diagram the prophage (white line) is shown completely inserted in the bacterial chromosome (black line). In the lower diagram only a short segment is inserted. In the former case, but not in the latter, an exchange between *b* and *c* would recombine the outside markers *S* and *T* (after Jacob and Wollman, 1959, in: Tunewall, *Recent Progress in Microbiology*, p. 15, Springfield, Ill., Charles C. Thomas).

prophage in lysogenic cells. Whether this is true of all reducible episomes is yet to be seen.

Only one prophage has been found in *E. coli* which does not segregate in crosses as would be expected if it were located on the chromosome. Since it is inherited maternally, always from the *F*⁻, it seems to have an extrachromosomal residence. It is interesting that this prophage yields the only type of phages that will nonspecifically transduce many different genes in strain K12.

Although most work on bacterial mating has been done with one strain of *E. coli*, K12, many other strains have been shown to undergo the process. It has even been possible to cross *E. coli* with *Shigella dysenteriae* and with *Salmonella typhimurium*, raising questions about the meaning of genus and species in such organisms. Furthermore, other bacteria, such as *Pseudomonas* and *Bacillus*, have been shown to mate, and so have the commercially important bacterial-like organisms, *Streptomyces*. In the last two cases, however, all strains do not show a pattern of recombinant types among the offspring that allows an interpretation of gene exchange in terms of crossing over or copy-choice mechanisms in a linkage group with genes in a linear order. Indeed, out of such matings come new genotypes that do not reflect the parental genes which entered the cross. Whether such phenomena are to be explained on the

basis of an intracellular transformation, or an irregular pairing of genetic material, or in some other terms, is yet to be seen.

These mechanisms of genetic exchange have become established during the period since 1940. The whole concept of infectious heredity is still novel, and new information is being obtained at a rapid rate. Certainly, basic discoveries about recombination are yet to come. The question of how similar will be the findings in the somatic cells of higher organisms is fraught with interest for such subjects as embryology, cancer, and senescence.

BIBLIOGRAPHY

- Adams, M. H., 1959. *Bacteriophages*. New York, Interscience Publishers, 592 pp.
- Burnet, F. M., and W. M. Stanley, 1959. *The Viruses; Biochemical, Biological and Biophysical Properties*; Vol. 1, *General Virology*, 609 pp.; Vol. 2, *Plant and Bacterial Viruses*, 408 pp.; Vol. 3, *Animal Viruses*, 428 pp. New York, Academic Press.
- Catcheside, D. G., 1951. *The Genetics of Micro-Organisms*. New York, Pitman, 223 pp.
- Demerec, M., Z. Hartman, P. E. Hartman, T. Yura, J. S. Gots, H. Ozeki, and S. W. Glover, 1956. Genetic studies with bacteria. *Carnegie Inst. of Wash. Publ.* 612, 136 pp.
- Ephrussi-Taylor, H., 1955. Current status of bacterial transformations. *Adv. in Virus Res.*, **3**:275-307.
- Hayes, W., and R. C. Clowes (ed.), 1960. *Microbial Genetics*. Society for General Microbiology, 10th Symposium. Cambridge, The University Press, 300 pp.
- Jacob, F., 1954. *Les bactéries lysogènes et la notion de provirus*. Monographies de l'Institut Pasteur. Paris, Masson et Cie, 176 pp.
- Lederberg J. and E. L. Tatum, 1946. Novel genotypes in mixed cultures of biochemical mutants of bacteria. *C. S. H. Symp. Quant. Biol.*, **11**:113-114.
- Luria, S. E., 1953. *General Virology*. New York, Wiley, 427 pp.
- Lwoff, A., 1953. Lysogeny. *Bact. Rev.*, **17**:269-337.
- Tunevall, G. (ed.), 1958. Recombination mechanisms in bacteria. In: *Recent Progress in Microbiology*, Symposium I, pp. 13-78, VII Inter. Congress for Microbiology, Stockholm, 1958. Springfield, Ill., Charles Thomas.
- Wollman, E. L., and F. Jacob, 1959. *La sexualité des bactéries*. Monographies de l'Institut Pasteur. Paris, Masson et Cie., 247 pp.
- Zinder, N. D., and J. Lederberg, 1952. Genetic exchange in *Salmonella*. *J. Bact.*, **65**:679-699.

6 What is a Gene?

The complete answer to the question, what is a gene, involves more detailed knowledge of nucleic acid chemistry and of the associations and actions of genetic material in the cell than we possess at present. A major share of the research efforts in genetics is nowadays directed at this question and our knowledge of the gene is considerably improved over what it was even a decade ago. But it must be appreciated at the outset that the word "gene" does not refer to a substance in the same way as does the word "water;" rather it refers to a concept as does the word "molecule," or "atom," or "electron." Although hereditary material consists of nucleic acid, a substance, the unit hereditary determinants are identified only on the basis of their biological activity. Consequently, these units can be defined only in an operational way; our concept of them is only a logical inference from a vast array of observations.

We have already pointed out how the hereditary unit which determines a specific function must be a polynucleotide in order to exist in enough different states to provide the coded information for all the manifold functions different genes perform. We also saw that even when single nucleotides were changed within the functional unit a mutation might result. As a consequence of such a change, the new functional unit is mutant when compared as a whole with the wild-type gene. How about the unit of transmission? Is it the equivalent of the functional unit or of the smallest subunit than can mutate? Or is it of some other dimension? Throughout most of the development of genetics it was assumed that the units of function, mutation, and transmission were the same indivisible structure. By considering new evidence which makes these concepts sharper, we will investigate the relations between them, attempting

always to remember the different experimental operations on which the concepts are based.

The gene was first thought of as a unit of heredity determining a difference in phenotype and detectable if it existed in two or more different states, which came to be called alleles, each with a characteristic developmental effect. This definition of the gene states that it is the ultimate *unit of function* responsible for biochemical activity and phenotypic difference; it is the "hereditary factor" of Mendel and the "gene" of the Danish biologist, Johannsen, who originated the word.

When an organism shows an inherited difference in character, we assume that this is due to the presence of an altered functional unit. We may distinguish between two nonallelic genes if one is responsible for a difference in one character and the other is responsible for a difference in another. Two recessive mutants, each arising independently from a wild-type strain, are considered to be due to allelic heritable units for the character in question if the homozygous recessive individual in which these units are combined has a mutant phenotype. It is assumed that there is a one-to-one correspondence between allelic genes and some basic cell function. If two allelic mutant genes carry out the same mutant function, a heterozygote between them cannot perform the normal wild-type function.

Functional units usually segregate at sexual reproduction in such a manner as to indicate that they are located on chromosomes. It is these functional units, and not extrachromosomal ones, which are the genes discussed in this chapter. Through crossing over or other exchange mechanisms, different units on a single chromosome may be separated from one another. In general, the closer together two units are, the less frequently will they be separated from one another by exchange. A single unit may or may not be separated during meiosis from other units at different locations on the same chromosome, but it will *always* be separated from its mate on the homologous chromosome (unless the homologous chromosomes do not disjoin, as is the case with attached X's in *Drosophila*). Such mated pairs, that always separate at meiosis, are called alleles. This definition of the *unit of transmission* depends upon our ability to observe the segregation of alleles in meiosis; alleles of the transmission unit *always* segregate from one another, nonallelic units *sometimes* segregate.

The distance between the loci of two nonallelic genetic units is measured in terms of the frequency with which they form recombinants. In practice, recombinants with frequencies as small as 10^{-6} have been observed. But theoretically, recombinants in much smaller frequencies would occur between units located closer together and could be detected

even if as infrequent as 10^{-8} , provided that mutations were so rare that they would not be confounded with recombinants. If in a cross no recombinants between two genes are found, it is still impossible to say that no recombinants would be observed if more offspring were examined. It is easier to define nonallelism than allelism in terms of the transmission unit, for the distinction between two very closely linked units (two pairs of alleles) and one unit (one pair of alleles) becomes a technical one, limited by our ability to analyze large numbers of progeny.

In addition there is the *unit of mutation*. Now, a chromosome carrying a mutant gene is in a sense a mutated unit and so is a mutant functional unit. It was in this broad sense that the mutable unit was considered in Chapter 2. But a more specific definition refers to that *smallest* part of the genotype which, when changed, is reproduced in the changed form. The transmission unit and the functional unit depend for their recognition on allelic differences initiated by mutation. Considerations from the chemistry of DNA lead us to believe that the functional unit and the smallest unit of mutation are not identical: there are other arguments which lead to the same conclusion.

When genes are not closely linked, the classical assumption that the three units are the same leads to no confusion: they mutate independently, their effects on the organism are distinguishable, and their recombination is frequent enough to observe. On the other hand, when two genes are closely linked, difficulties with the classical concepts arise. First, there do occur simultaneous mutations of recombinable genes with separate functions; furthermore, genes that mutate independently and have distinguishable effects may not recombine; finally, genes that mutate independently and recombine may have indistinguishable functions. In the first case, the unit of mutation seems larger than the other two; in the second case, the transmission unit, and in the third case, the functional unit, seem to be largest.

Simultaneous mutation of closely linked genes does not necessarily demonstrate the existence of a mutable unit containing smaller functional and transmission units, for it could be due to the simultaneous mutation of two neighboring genes, brought about, for example, by a minute deletion or chromosomal rearrangement. The independent mutation of different functional units in a chromosomal region where crossing over has not occurred does not necessarily mean that it cannot occur. Such genes may be very closely linked or some parts of the chromosome may cross over less frequently than others of equal physical size. But when units that mutate independently and recombine appear to be allelic, we may be confronted with the situation called *position pseudo-allelism*.

POSITION PSEUDOALLELISM

Evidence for this phenomenon has been found repeatedly in higher forms, especially in *Drosophila*, where it was first detected and extensively studied by E. B. Lewis. In this fly, for example, there is a region in the first chromosome, about 0.2 of a map unit in length, in which are located at least three genetic units called lozenge (lz). They have, in mutant form, similar effects on the morphology and pigmentation of the eyes. Each factor is recessive when present singly, e.g.:

$$\begin{array}{ccc} lz_1 & + & + \\ + & + & + \end{array} \quad (\text{normal})$$

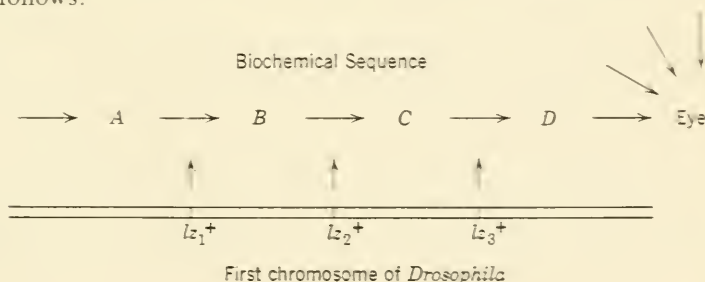
But in heterozygotes with two different mutant factors the effect depends upon whether the normal units are on the same chromosome (*cis*-arrangement), e.g.,

$$\begin{array}{ccc} lz_1 & lz_2 & + \\ + & + & + \end{array} \quad (\text{normal})$$

or whether one normal factor is on one chromosome and the other on its homologue (*trans*-arrangement), e.g.,

$$\begin{array}{ccc} lz_1 & + & + \\ + & lz_2 & + \end{array} \quad (\text{mutant})$$

In the former case the phenotype is normal: in the latter case it is mutant. Thus, for normal function there must be one set of normal factors on at least one chromosome. There is not only a position effect but it is such that a *trans*-heterozygote for two independently mutable and recombinable units shows a mutant character. We may ask whether it is possible in this case to be sure that the genetic subunits in this complex form one large functional gene which can operate only when all of its parts are normal. This question is especially difficult to answer in an organism such as *Drosophila* in which the biochemistry intervening between the gene and a character such as the eye is not well understood. Alternatively, each lz^+ unit may be a separate gene and perform a different primary function, and these functions may be linked in space and time as follows:



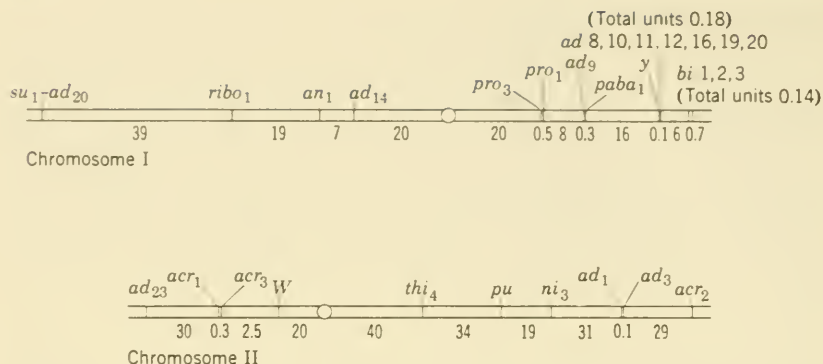


FIGURE 6.1. Linkage maps of two of the chromosomes of *Aspergillus nidulans*. Chromosome I is drawn on twice the scale of Chromosome II (after Pontecorvo, 1958, *Trends in Genetic Analysis*, New York, Columbia University Press).

The sequence may not pass from one chromosome strand to another because of special properties of substances *B* and *C*.

In microorganisms, on the other hand, the intimate biochemical functions of genes are sometimes better understood than in higher forms, and it might be expected, therefore, that pseudoallelism could be studied more conveniently by using them. Some cases of close linkage between genes with similar functions or concerned with related steps in the same synthesis have been found in microorganisms and analyzed with informative results.

In the fungus, *Aspergillus nidulans*, intensively studied by Pontecorvo and co-workers, chromosome II can mutate at two closely linked sites called ad_1 and ad_3 , either of which causes a requirement for adenine (Figure 6.1). In crosses through the heterozygote $\frac{ad_1^+}{+ ad_3}$, recombination occurs in 0.1 per cent of the cases, one of the products being the adenine-independent form, $ad_1^+ ad_3^+$, which can be found in low frequency by selection on minimal medium. Although ad_1 and ad_3 are indistinguishable by nutritional tests, both growing on adenosine, adenine, hypoxanthine, or 4-amino-5-imidazole carboxamide, they complement one another and allow the growth of a *heterokaryon*, which carries nuclei of both types in a common cytoplasm $\frac{ad_1^+}{+ ad_3}$. These adenineless genes are functionally different, mutate independently, and recombine. They are as distinct as loosely linked nonallelic genes such

as ad_1 and ad_{23} , or closely linked nonallelic genes with clearly different functions such as yellow (y) and ad_8 on chromosome I. The ad_1 and ad_3 genes are clearly nonallelic on all three definitions of the gene.

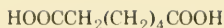
It is otherwise with the seven adenineless mutants in the ad_8 group on chromosome I. They, like the ad_1 and ad_3 mutants, are nutritionally indistinguishable, growing on the same compounds. None of the group, however, will complement any other of its members, either in the heterokaryotic condition or when heterozygous diploids are formed (Chapter 12). Functionally they behave as alleles, yet, when intercrossovers are made, adenine-independent nuclei arise during meiosis with frequencies that cannot be accounted for by back mutation as it occurs in haploids. These recombinations locate the seven mutational sites in sequence between the y and biotin (bi) markers which are only 6 map units apart. The order is determined pairwise in this fashion: from the heterozygote

$\frac{y}{+} \frac{ad_8}{ad_{16}} + \frac{+}{bi}$ there were 139 sexual spores which gave cultures showing adenine independence; of them, 133 were yellow and six were green (Y^+), suggesting the order $y:ad_{16}:ad_8:bi$, in which case the composition of the heterozygote should have been written $\frac{y}{+} + \frac{ad_8}{ad_{16}} + \frac{+}{bi}$. The

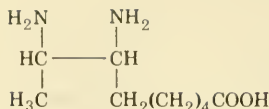
segregation of the less closely linked bi confirmed this qualitative conclusion. When exchanges occur between these mutants, the reciprocal recombination products may be derived from single nuclei (Chapter 12), showing that the process meets the formal requirements of crossing over.

Considering for illustrative purposes interactions between just two of these mutants, the results can be summarized as in Figure 6.2. It can be seen that when either ad_{16} or ad_8 is present alone in a heterokaryon or a heterozygote, it is recessive and gives a normal wild-type phenotype. The phenotype is normal whenever the two dominant factors are on the same chromosome (*cis*); but when one dominant factor is on one chromosome and the other on its homologue (*trans*), the mutant character is expressed. Thus ad_{16} and ad_8 behave as functional alleles in this instance, but they seem to occupy two separable sites as do nonallelic genes. The contrast can be brought out by considering the gene y which is closer (0.048 units) to ad_{16} than the latter is to ad_8 (0.14 units). Yet where ad_{16} and ad_8 behave as pseudoalleles, ad_{16} and y do not; the *trans*-heterozygote $\frac{y}{+} + \frac{+}{ad_{16}}$ is wild type.

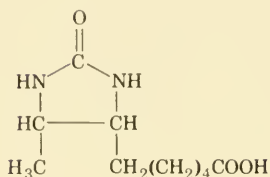
The bi factors at the adjacent locus give another example. All three will grow on 7,8-diaminopelargonic acid, desthiobiotin or biotin, but not on pimelic acid.



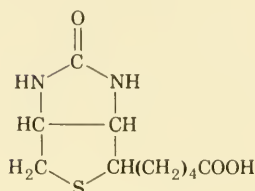
Pimelic acid



7,8-Diamino pelargonic acid



Desthiobiotin



Biotin

Furthermore, they do not cross-feed one another, nor will stocks carrying one *bi* gene form biotin-independent heterokaryons with stocks carrying another. In this way the *bi* factors behave as functional alleles of one another. They are, however, separable as is shown by the fact that in crosses through the trans-heterozygote $\frac{biA}{+} \frac{+}{biB}$ they recombine to give the $biA^+ biB^+$ biotin-independent type which can be selected for on minimal medium.

The frequencies of recombinants indicate that they are arranged in this order between the *y* and the *ad*₄ loci:



The behavior of the *y* and *ad*₄ markers in crosses yielding $biA^+ biB^+$ types is compatible with their exact locations on chromosome I as determined in other crosses. This indicates that the $biA^+ biB^+$ recombinants arise by a process of crossing over.

It is their behavior in heterokaryons and in heterozygous diploid nuclei that differentiates the *bi* factors in behavior from *ad*₁ and *ad*₃, and makes them resemble the factors in the *ad*₈ group. In *trans*-configuration the mutant phenotype is expressed. They behave, therefore, as pseudoalleles.

There are two reasonable explanations of this phenomenon. The first presumes that chromosome I has a segment 0.1 map unit long which functions at some step in the conversion of pimelic acid. This segment has at least three mutable components which can be separated by cross-

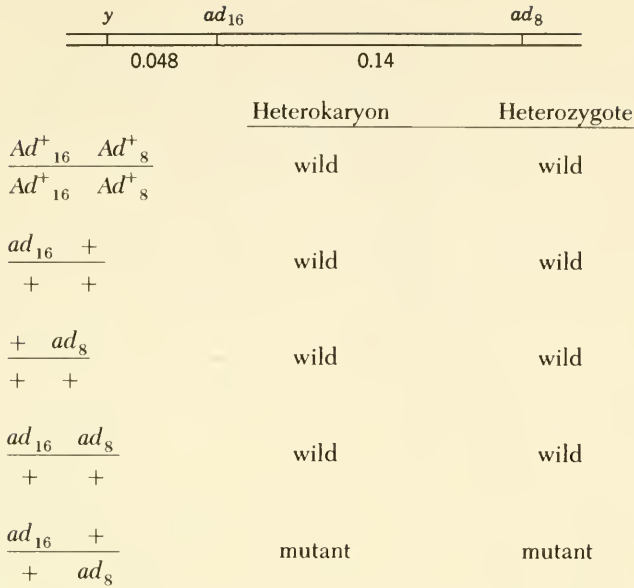
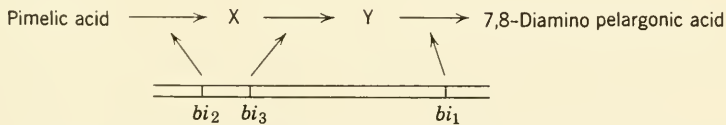


FIGURE 6.2. Map of two mutants at the *ad*₈ locus of *Aspergillus nidulans* and the effect of *cis* and *trans* arrangements in heterokaryons and heterozygotes.

ing over and these are recognized as *bi*₂, *bi*₃ and *bi*₁, in that order. When any one of these units is mutant, the functional gene of which it is a part cannot operate normally. Hence the *trans*-heterokaryon and *trans*-heterozygote fail. In the *cis*-configuration there is on one chromosome an array of normal units, and this will function in the synthesis of the pelargonic acid. In other words, the functional unit is large and contains several mutable and recombinable subunits.

The alternative hypothesis contends that each of the *bi* genes has a separate but related function. The production of some labile or non-diffusible substance, or of some substance in millimicromolar amounts, might require that the genes need be located next to one another on the same chromosome in order that the sequence of reactions be carried out. For example:



Hence, all *trans*-combinations would fail, including the heterokaryon test, because the products of one *bi* gene may not be transported to the

TABLE 6.1

Results of Transducing *try D-11 cys B-18* Bacteria
with Phages Grown on *try B-2* Bacteria. After Similar Samples Were Plated
on Different Media, They Were Replicated to Other Media

(From Demerec, 1956, in *Mutation*, Brookhaven

National Laboratory Symposium, p. 75)

Medium	Pheno- type	Number of Colonies	Region of Exchange if Order is:											
			+ <i>try B</i> +				+ + <i>try B</i>				<i>try B</i> + +			
			1	2	3	4	1	2	3	4	1	2	3	4
			<i>try D</i>	+	<i>cys B</i>		<i>try D</i>	<i>cys B</i>	+		+	<i>try D</i>	<i>cys B</i>	
Minimal	+	14	1	2	3	4	1		3			2		4
Cystine	+	8												
	<i>cys</i> ⁻	70	1	2			1	2				2	3	
Indole	+	17												
	<i>ind</i> ⁻	628	1			4	1				4	1		4
Tryptophane	+	12												
	<i>ind</i> ⁻	659												
	<i>try</i> ⁻	2381	3-4	2-4			2-3	2-4			3-4	1-2-3-4		
Cystine	+	14												
indole	<i>cys</i> ⁻	110												
	<i>ind</i> ⁻	543												
	<i>cys</i> ⁻ <i>ind</i> ⁻	1460	1		3		1	2	3	4	1		3	

next. This hypothesis assumes that the recombinable and functional units coincide.

THE STRUCTURE OF GENES IN *SALMONELLA*

Demerec and his colleagues have made an enlightening analysis of functional units in *Salmonella typhimurium* by the use of transducing phages. A large number of mutants concerned with certain biochemical reactions were obtained; among these, some 450 required histidine and 77, tryptophane. These mutants could be transduced to prototrophy at a certain frequency when infected by phages grown previously on wild-type *Salmonella*. Within any group of mutants, individual members could be transduced by phages grown on another member, but always at lower frequencies than when the phages were grown on wild type. Transduction to prototrophy presumably occurs by recombination between the host chromosome and a fragment of the donor genetic material. Consequently the frequency of wild-type progeny may be a measure of the degree of linkage—the lower the frequency the greater the linkage. All members of a group of mutants can be subgrouped on

the basis of transduction frequencies and these subgroups have been found to coincide with the biochemical properties of the mutants. For example, the 77 tryptophane mutants fall into four groups: *A*, members of which grow on either tryptophane, indole glycerol phosphate, indole, or anthranilic acid; *B*, which grow on either tryptophane, indole glycerol phosphate or indole and accumulate anthranilic acid; *C*, which grow on either tryptophane or indole, but accumulate indole glycerol phosphate; and *D*, which will grow only on tryptophane. All subgroups are linked with a cystine *B* locus and seem to be located in the order *cys B:try A:try B:try D:try C*.

This order can be confirmed by making three-point crosses via transduction, in which a closely linked cystineless locus is utilized. Since the nutritional deficiencies of members of the various groups differ, it is possible to detect the various recombination classes. As an illustration of the method, results obtained by employing *try B-2 try D-11*, and *cys B-18* are presented in Table 6.1. The size of the *cys⁻ ind⁻* class eliminates the order *try D:cys B:try B*, which would require four exchanges. In reciprocal experiments where phages from *try D-11 cys B-18* bacteria were used to transduce *try B-2* bacteria, the results shown in Table 6.2 were obtained. The order *try B:try D:cys B* is eliminated by the size of the *cys⁻* class which requires a quadruple exchange. Thus we can conclude the order is *try D:try B:cys B*. By means of similar experiments the order was found to be that shown in Figure 6.3.

The linkage order is here the same as the order of reaction sequences; yet at least the functional units *B*, *D* and *C* are further subdivisible. A more extensive subdivision of functional units is to be found among the alleles for histidine synthesis, analyzed in a similar fashion and arranged as shown in Figure 6.4. Once again, the functional units are

TABLE 6.2
Results of Transducing *try B-2* Bacteria with
Phages Grown on *try D-11 cys B-18* Bacteria
 (From Demerec, 1956, in *Mutation*, Brookhaven
 National Laboratory Symposium, p. 75)

Medium	Pheno- type	Number of Colonies	Region of Exchange if Order is:							
			<i>try D</i> + <i>cys B</i>				+ <i>try D cys B</i>			
			1	2	3	4	1	2	3	4
			+	<i>try B</i>	+		<i>try B</i>	+	+	
Minimal	+	491		2	3		1	2		
Cystine	+	399								
	<i>cys⁻</i>	731		2		4	1	2	3	4

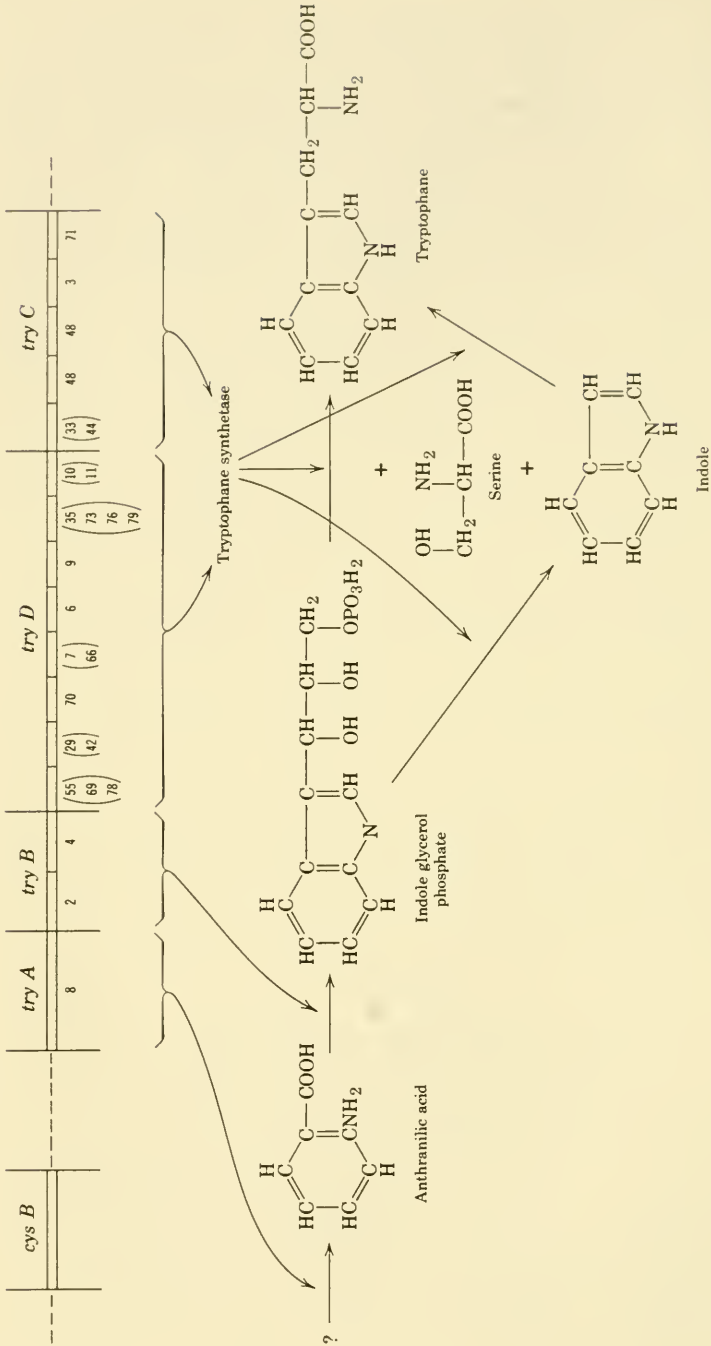


FIGURE 6.3. Genetic map of a series of tryptophaneless mutants of *Salmonella typhimurium*. Actual distances are not shown (from Demerec, 1956, Genetic studies with bacteria, Carnegie Inst. Wash. Publ. 612 and Balbinder, unpublished).

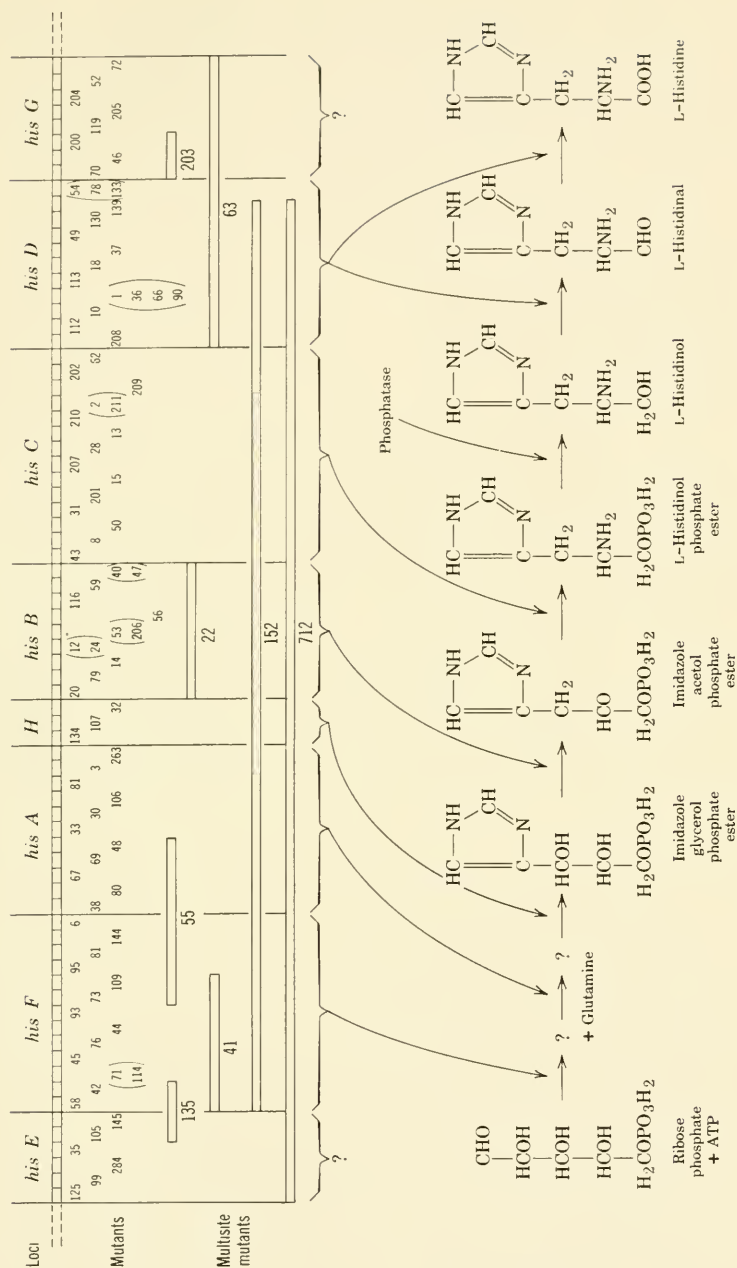


FIGURE 6.4. Correlation of the genetic map of a series of histidineless mutants of *Salmonella typhimurium* with the reaction sequence in the synthesis of histidine. Only those mutants whose order is known are shown in the various loci. The map is not drawn to show quantitative relations (adopted from Hartman, Loper, and Sërman, 1960, *J. Gen. Microbiol.*, **22**:323).

linked in the same order as the reaction steps with which they are concerned, and yet each unit is further subdivisible by mutation and recombination. Some recombinable genes cannot transduce one another and therefore seem to reside at the same site. A different situation is found with mutants such as 22, which cannot transduce other members of its group, although they can transduce one another. Mutant 22 seems, therefore, to have a location that covers the other members of group B. It could be a deletion. The same reasoning has been applied to the other mutants shown as bars rather than points in Figure 6.4.

Although the bacterial cell is haploid, it is possible to test the behavior of the mutable units in *cis*- and *trans*-configuration by using abortive transduction. A majority of the transductions do not involve incorporation of the genetic material of the donor into the reproducing genetic system of the recipient. The transduced fragment exerts its influence on the host's phenotype but is passed to only one of its progeny. This constitutes a line, and on minimal medium a tiny colony is the result (see Figure 5.12). Streaking such small colonies on minimal agar results in the formation of only one colony, that formed from the cell which inherited the transduced fragment. Whenever a transduction experiment is carried out between mutants in different segments, abortive transductions result, e.g., $\frac{his\ C-8}{+} + \frac{his\ D-18}{+}$. Since the nonmutated seg-

ments complement one another, they behave as independent functional units; they meet the *cis-trans* test for allelism. It is otherwise when transductions are performed between different mutants in the same segment. There abortive transductions fail to occur since *trans*-arrangements within a functional unit, such as $\frac{his\ D-10}{+} + \frac{his\ D-18}{+}$ fail to complement each other. On this basis they appear as different defects in the same functional unit, neither of which, as a consequence, can enable the formation of histidine from histidinol. The functional gene is clearly subdivisible into smaller mutable and recombinable units.

The discovery that in *Salmonella* loci concerned with sequential steps in a biosynthesis are arranged in that order on the chromosome came as a surprise, because such linkages had not been found in *Aspergillus* and *Neurospora*. We can understand the finding in the following way. Neighboring genes may arise as duplications or repeats of a part of the chromosome. One or more of these repeats may gradually differentiate to assume a function related to but not identical with the original one, becoming thereby functionally nonallelic to its neighbor. This idea has much theoretical attraction, including the observation that the product of one gene is the substrate of its neighbor. These differentiated dupli-

cations will remain linked on the chromosome only if there is some selective value to the association; otherwise we would expect them to be eventually broken up through chromosomal rearrangements. Repeats need not remain linked on the chromosome if the necessary organization of their products can be achieved at some functioning cytoplasmic site. *Salmonella* and *E. coli*, in which similar linkages have been found, may not depend on cytoplasmic particles for their heterocatalytic syntheses. Rather, the genes may be more directly involved, either by acting as catalysts themselves or by retaining in their immediate vicinity the templates or enzymes which they produce. The organized synthesis may, in bacteria, take place on the genetic material itself, the organization of the chromosome being maintained by selection. On the other hand, it may be that in bacteria, where chromosomal rearrangements have not yet been demonstrated adjacent repeats are not usually disorganized through translocation and inversion.

How does all this relate to the explanation of pseudoallelism in terms of the two hypotheses proposed? Even genes linked in the order of the sequence of reactions they control give rise to the wild, not the mutant, phenotype when brought together in a diploid. Their wild-type alleles need not be located on the same chromosome for their products to interact. Furthermore, the evidence indicates that the functional gene is subdivisible by mutation and recombination; this is a sufficient explanation of the position effect observed with pseudoalleles. But how general is this phenomenon? It has been observed in yeast and fungi and probably in higher forms. But the most extraordinary analysis of the fine structure of the gene has been made in bacteriophage.

THE STRUCTURE OF THE GENE IN BACTERIOPHAGE

In the *T4* bacteriophage of *E. coli* there are a number of closely linked genes in the *r* region on the map shown in Table 5.2. These so-called *rII* genes have the function of allowing phages grown on strain *B* to multiply normally on strain *K12* after infection; mutations in these genes prevent the phages from growing on strain *K12*. By infecting strain *B* with equal numbers of two different *rII* mutants and plating the new phages produced after lysis on strain *K12*, their relative positions in the linkage group can be determined. If a recombination has occurred and one of the recombinants contains the two normal alleles, it will lyse the *K12* bacteria and form a plaque. The ratio of plaques on *K12* to the count on *B* measures the frequency of recombinants. Infection of strain *B* with phages of a single *rII* type results in the production of only very

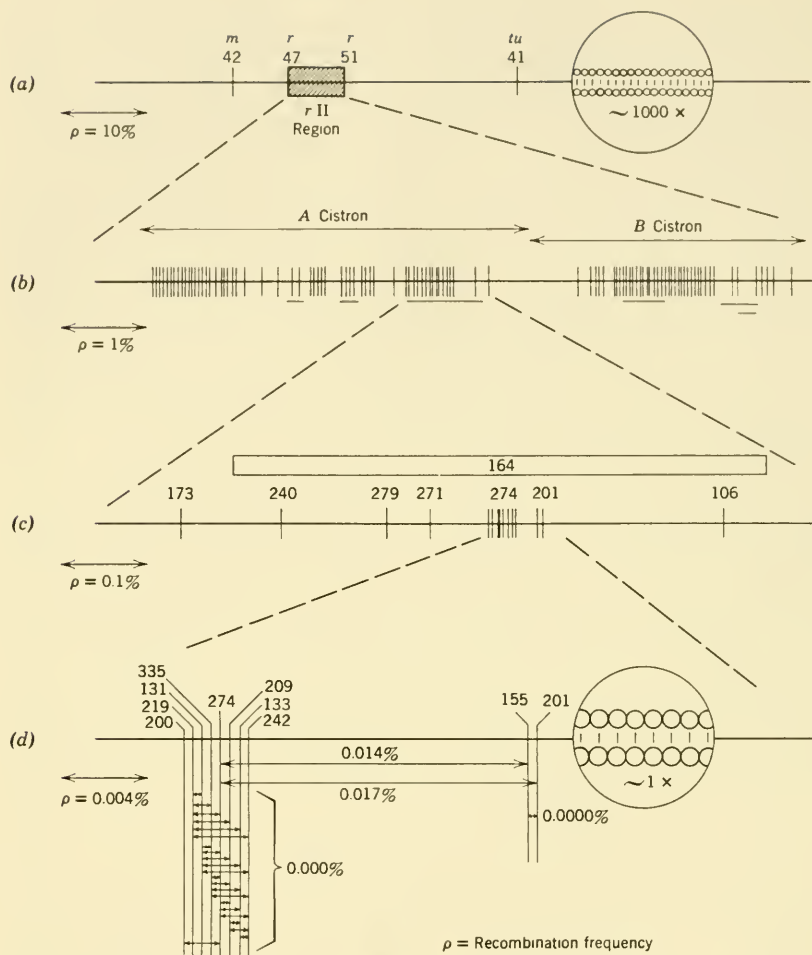


FIGURE 6.5. Linkage maps of T4 phage (from Benzer, 1956, *Mutation, Brookhaven Symp. Biology*, 8:3).

(a). The location of the *rII* region is shown. The inset shows the estimated corresponding spacing of nucleotides (circles) according to the structure of Watson and Crick on the assumption that the phage "chromosome" is such a fiber of DNA.

(b). The locations are shown of a number of *rII* mutants within the region. (There is some distortion due to the difficulty of drawing lines sufficiently close to one another.) A horizontal line represents an "anomalous" mutant which produces no wild recombinants in crosses with any mutant covered by its span.

(c). A selected group is shown on larger scale.

(d). Further magnification.

few wild-type phages attributable to back mutation. Quite a number of *rII* mutational changes have been located on the phage chromosome in this way (Figure 6.5).

Some mutants such as *r164* seem to involve changes that extend for some distance, overlapping a number of others. These will not produce recombinants with any of the mutants whose positions they overlap; for example, mutant 164 will give wild-type recombinants only with mutant 173 among all those shown in Figure 6.5c. Because they do not revert, they are judged to be deletions. They proved of practical value in allowing a short-cut in mapping almost 1000 independent mutants. This was necessary because pairwise matings of 1000 mutants would require $1000 \times 999/2$ crosses, a task of heroic dimensions. The short cut consisted of crossing the mutant to be located with a series of broad deletions (Figure 6.6). If the cross did not yield wild-type recombinants, the defect could be assigned to one of the segments. For example, if a mutant gives wild-type recombinants with all but *rH88*, *rA105*, and *r164*, its defect must be located in segment 4. The one which gives recombinants with all must have its defect in segment 1. Segments 7, 9, and 11 are not distinguishable by the set of mutants shown in Figure 6.6. In this way, then, many unnecessary crosses can be avoided.

If strain *K12* is mixedly infected with wild-type phages and an *rII* mutant, $\frac{47}{+} \frac{+}{+}$, the wild type supplies the function lacking in the mutant and the bacteria lyse, liberating both types of phages. The *trans*-configuration is simulated by infecting strain *K12* with a pair of *rII* mutants. If both mutants belong to the same segment, *A* or *B*, e.g.,

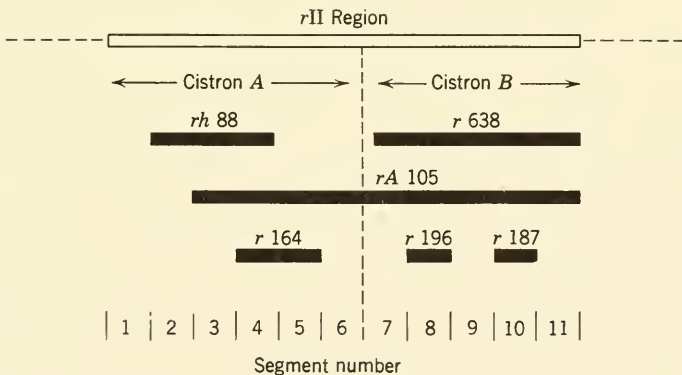
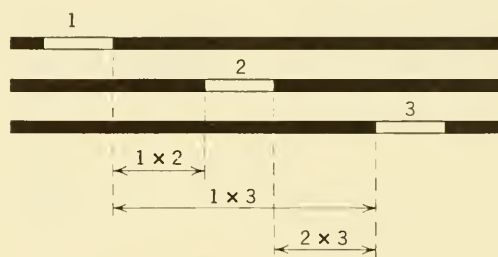


FIGURE 6.6. Arrangement of a number of extensive defects (deletions) at the *rII* locus of phage T4 (after Benzer and Freese, 1958, *Proc. Natl. Acad. Sci.*, 44:112).

$\frac{47}{+} \frac{+}{104}$, mixed infection gives the mutant phenotype, and very few cells lyse. If the two mutants belong to different segments, A and B, e.g., $\frac{47}{+} \frac{+}{51}$, extensive lysis occurs, yielding both types of phages and recombinants. Therefore, the two segments of the *rII* region correspond to different functional units but the units within each segment behave as pseudoalleles.

It is clear that genes as functional units are large and contain many smaller mutable units separable by recombination. An estimate can be made of the size of these units. The smallest recombinable unit, called a *recon*, is no larger than the smallest observed nonzero recombination value; it is around 0.02 map units in length. Similarly, the size of the *muton* can be estimated if it is defined as the smallest element, alteration of which can be effective in causing a mutation. One method involves observing the number of mutational changes that can exist within a given map length. Nine separable changes were located within a distance of 0.8 map units setting an upper limit of 0.09 map units for the muton. Another method consists of measuring the distance between the sites of two peripheral mutational alterations and comparing it with the sum of the distances between each of these and an altered site in between them, e.g.,



With this method it was shown that the muton must be smaller than 0.05 map units.

Benzer, who is responsible for this line of analysis, has attempted to translate these abstract genetic map constructions into a physical map measured in terms of the number of nucleotide pairs in a DNA double helix. The DNA content of phage *T4* is ca. 4×10^5 nucleotides. From the star experiments (Figure 5.2) it seems that 40 per cent, or 1.6×10^5 nucleotides make up the genetic material.^o If there is just one copy of genetic information and it has the paired structure of the model of Watson and Crick, the total length of the hereditary material should be

^oBut see footnote on p. 117.

8×10^4 nucleotide pairs. There seems to be just one linkage group in phage T4 with a length of about 800 map units, corrected for successive rounds of mating and for interference. Dividing 800 map units by 8×10^4 nucleotide pairs gives 0.01 map units per nucleotide pair. That is to say, if two mutants, having mutational changes one nucleotide apart, are crossed, the proportion of recombinants in the progeny should be 0.01 per cent.

This is a very exciting result for the maximum sizes of the recon and of the muton approach very closely the length of one nucleotide pair. We should recall the evidence on induction of mutation in TMV by nitrous acid, where a change in one nitrogen base seemed capable of causing a mutation. Nonetheless, there are a fair number of assumptions in the calculation of muton and recon size. It was gratuitously assumed, among other things, that the probability of recombination per unit molecular length was constant and that the vagaries of interference were appropriately assessed. Without doubt, as our knowledge becomes more refined, such calculations will be improved, but it now seems probable that revision will be in the direction of establishing the muton as a single nucleotide pair. This is what we considered it to be in the discussion of mutation (Chapter 2). Recombination presumably occurs between sequential couplets of nucleotide pairs. But whether it does so by actual breakage and reformation of the sugar-phosphate backbone of DNA or by some replication mechanism is another question.

A CONCEPT OF THE GENE

The functional unit is certainly the complex one. On the basis of calculations like those made above, it should consist of hundreds to thousands of nucleotide pairs. Its identification by the *cis-trans* test allows the sharpest definition of its limits, even when the enzymology of its effect is unknown. As a consequence, it is named the *cistron*. But even the test of complementation is not absolute, and results of the *cis-trans* test must always be scrutinized critically. Cases are known where mutons within the same cistron *partially* complement one another. One instance is found in *Aspergillus* where, at the ad_9 locus on chromosome I, there is a cistron containing six identified mutational sites. Some of these are noncomplementary, e.g., $\frac{ad_{13} +}{+ ad_{15}}$. But the *trans*-configuration $\frac{ad_{17} +}{+ ad_{15}}$ grows a little on minimal medium, showing a partial complementation. In another case, two *Neurospora* mutants affecting the

synthesis of glutamic dehydrogenase complement one another in heterokaryons. So do a number of mutants affecting the synthesis of adenylosuccinase in the same organism. In the latter case the more peripheral sites show the greatest complementation. When the enzyme contents of these *trans*-heterokaryons are measured, there is only a partial restoration of activity; at the most only 25 per cent of the wild-type activity is attained. Such intra-allelic complementation is probably achieved by the interaction between gene products. Isolation of nuclei from heterokaryons in which sites in the same cistron had been interacting in *trans*-configuration, shows that their genetic structure is unchanged. In higher forms, similar complementation exists between mutons within what seems to be a cistron. These can be fragmented only rarely, if ever, by recombination. The series of *t* sites affecting the development of the tail and hind parts of mice is a case in point.

Thus when two mutant functional units that are nonallelic are confronted in transposition, complete complementation results; but when two allelic functional units are confronted in transposition, only partial complementation results at best. As long as this distinction holds in practice, the functional unit can be operationally defined in terms of the *cis-trans* test. But since the amount of partial complementation may increase up to 25 per cent in proportion to the distance between the mutant sites within the functional unit, we may ask whether a fourth unit exists, smaller than a functional unit but larger than a recon and a muton. In this event, there should be a cluster of mutons which complement to the same degrees other groups located elsewhere within the functional unit. The evidence available at present does not allow a certain distinction between such a discontinuous arrangement along the functional unit and a continuous gradient in the quantity of complementation with distance. Elucidation of the intimate mechanism of intragenic complementation promises a better understanding not only of gene action but also of gene structure; it is further discussed in Chapter 10.

We can meanwhile construct an imperfect image of the gene. More than one may be possible and the concept we will present is only an approximation, accounting for a vast assemblage of facts. Its greatest virtue is its specific nature, wherein also lies its greatest tendency for error. But being specific, it is amenable to test by experiments which will allow its proper modification in time.

The cistron is considered to be a coded sequence of nucleotide pairs in a DNA molecule. It must be larger than a muton and a recon, otherwise single nucleotides could function as genes. It may carry the information necessary for the specification of the order of amino acids in

large polypeptide chains. That part of the cistron that specifies the location of a single amino acid is in a sense a functional unit and points up again the series of hierarchies of organization which exist within the aperiodic DNA crystal. If the function we measure is the result of some enzyme action, the functional unit which underlies it must have an extent and complexity sufficient to determine the order of amino acids in a polypeptide large enough to show that enzyme activity. The best estimate from *cis-trans* tests of the *rII* mutants in phages indicates that the cistron has dimensions of the order of 10^3 nucleotide pairs.

The base sequence within a cistron has not yet been analyzed, but an internal differentiation has been revealed indirectly. Multiplying *T4* phages have been treated with mutagens such as 5-bromouracil, 2-aminopurine, nitrous acid, heat and low pH. The sites of the mutations produced at the *rII* locus show a spectrum peculiar to each mutagen and different from the distribution of sites that mutate spontaneously (Figure 6.7). This arrangement of so-called hot spots indicates a nonhomogeneity along the length of the cistron. It is known that 5-bromouracil specifically replaces thymine in DNA. On the other hand, 2-aminopurine seems to replace both adenine and guanine. The pattern of mutations induced by these substances is a reflection of the chemical heterogeneity along the two DNA polynucleotides that constitute the cistron. This method, in principle, promises a way to a base analysis of genetic material.

Another example of heterogeneity is found in the rates at which

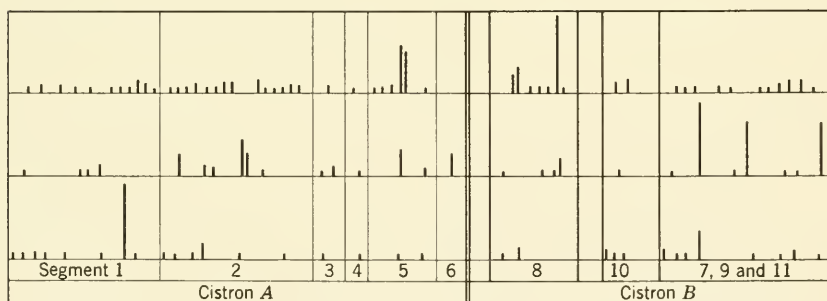


FIGURE 6.7. Genetic maps showing the locations of spontaneous, 5-bromouracil- and 2-aminopurine-induced mutational alterations in cistron *B* of the *rII* region of phage *T4*. The height of each column indicates the number of mutants. The occurrence of one mutant in the induced sets reflects a much higher mutability than in the spontaneous set. As a consequence, the background of spontaneous mutations and mutations occurring with low frequency do not show up on the maps of induced mutants (from Freese, 1959, *Proc. Natl. Acad. Sci.*, **45**:622).

TABLE 6.3

**Average Numbers of Revertants Found Among 10^6 Mutants Altered at
Various Sites within a Single Cistron**

(After Freese, 1959, *Proc. Nat. Acad. Sci.* **45**:622, and Streisinger and Franklin, 1956, *C. S. H. Symp. Quant. Biol.*, **21**:103)

Phage T4	$rII \rightarrow rII^+$	Phage T2	$h^+ \rightarrow h$
$r111$	283	h^+11	0.3
$r114$	0.20	h^+23	0.3
$r117$	1.7	h^+10	0.3
$r131$	1.2	h^+37	0.5
$r157$	763	h^+0	1.0
$r215$	0.002	h^+34	1000
		h^+41	1000

various mutable sites will revert from the recessive state to wild type. Table 6.3 indicates that these rates may vary ten-thousand-fold. When the revertants were crossed with wild types, no mutants were found among sufficient progeny to indicate that the reverted site was within 0.1–0.2 map units of the original mutant site; these reversions are either true back mutations or they are due to very closely linked suppressors. The rates of forward mutation from wild type to the mutant state shown in the table are so high as to make the frequency of mutants among the r^+ or h parents ca. 10^{-4} . This suggests that there is only one state of the DNA in each of these cistrons that yields the r^+ and the h phenotypes, and that the various sites listed in the table are located within these cistrons. Since each of these sites, when mutant, impairs the function of the cistron and causes the phenotypes r or h^+ , the rates of mutation from $r^+ \rightarrow r$ and $h \rightarrow h^+$ are the sums of the rates of change of many mutons and, as a consequence, are high. The rates of reversion, which determine the frequencies of mutants shown in Table 6.3, are, on the other hand, unique for the rate with which a particular site changes back to that one condition which allows the cistron to function. Since there are numerous classes of reversion rates, the stability of a muton must involve more than the likelihood of a substitution of one of four bases by another. Even if all changes were caused by base substitutions only, the chance of such an event would be determined by the nature of the neighboring bases or other structural features of the polynucleotide itself. All this speaks for a considerable chemical heterogeneity within a functional unit.

Even within a segment of the gene where no recombination has been observed, a variety of changes can occur which differ in stability.

For example, 123 mutants have been shown to result from alterations at the *r274* site of the *rII-A* cistron of phage *T4*. These can be divided into three groups according to the frequency of reverted r^+ among them: *alpha* group, $0.2\text{--}0.9 \times 10^{-6}$; *beta* group, $0.3\text{--}4 \times 10^{-3}$; and *gamma* group, $0.5\text{--}1 \times 10^{-8}$. These differences may be due to alternate structures of the site or to the fact that what seems to be one mutable site is really composed of at least three mutons. We saw that the *r274* site, like others, could not be larger than 0.05 map units; if this is divided by three, we obtain 0.02 units, which is indistinguishable from the estimated genetic length of one nucleotide pair.

In addition to differences in stability, sites within a single gene may show other specific differences. For example, although suppressors of a given locus are frequently nonspecific in their action, some are highly specific. The *ad*₂₀ mutant in *Aspergillus* is the only one of the *ad*₈ group responsive to a suppressor in the left arm of the same chromosome. Although many recessive mutants form no enzyme peculiar to the cistron, some are "leaky," their defect allowing the synthesis of normal enzyme at a slow rate or of an enzyme with reduced activity. Still others form enzymatically inactive proteins with serological relationships to the enzyme. Frequently a completely nonfunctional mutant will revert to a state of partial activity. Then again, some alleles, such as the *h*⁺ series in *T2*, cause the phages containing them to be inactivated by heat at different rates.

Clearly, on the criteria of stability and function, a functional unit may achieve many alternate states which we recognize as multiple alleles. Each allelic stage represents a particular configuration of the gene in terms of the mutons it contains. Since there may be hundreds of mutons in a gene, the number of possible alleles must be very large. Many alleles which are really different may be indistinguishable because of the insensitivity of the tests we use to discriminate among them. When discrimination can be achieved only by changing the genetic background or by using special environments, the alleles are called *isoalleles*. A most favorable case for the discovery of different alleles is found in the incompatibility locus of some higher plants. Unless the male and female gametes carry different alleles of this locus, fertilization will not succeed. The more different incompatibility alleles, the more likely the success of a random pollination. Clover, as an example, seems to have exploited many of the changes that can occur at its incompatibility locus, for more than 200 alleles have been discovered there.

When a multiple allelic series in, say, *Drosophila*, is carefully studied by recombination techniques, it is usually found that the locus is pseudo-allelic. This simply means that it is a gene composed of a number of

TABLE 6.4
Smallest Distances Measured in Seven Organisms,
Expressed as Fractions of the Total DNA
 (After Pontecorvo, 1958, *Trends in Genetic Analysis*.
 New York: Columbia University Press, p. 25)

Organism	Total Linkage Map Units	Total DNA in Nucleo- tide Pairs	Nucleo- tide Pairs per Map Unit	Minimum Distance Measured in Map Units	Minimum Distance Measured as Fraction of Total Map	Minimum Distance Measured in Nucleo- tide Pairs
Phage T4	800	8×10^4	1×10^2	2×10^{-2}	2.5×10^{-5}	2
<i>E. coli</i>	2000	1×10^7	5×10^3	2×10^{-3}	1×10^{-6}	10
<i>Asper- gillus</i>	660	4×10^7	7×10^4	1×10^{-4}	1.5×10^{-7}	7
<i>Neuro- spora</i>	302	4×10^7	1×10^5	1×10^{-3}	3×10^{-6}	100
<i>Droso- phila</i>	280	8×10^7	3×10^5	8×10^{-4}	2.9×10^{-6}	24
Mouse	1954	5×10^9	3×10^6	2×10^{-1}	1×10^{-4}	600,000
Corn	1350	7×10^9	8×10^6	2×10^{-1}	1.5×10^{-4}	1,600,000

subunits. The word "allele" may be used to refer to alternate states of the whole gene, or it may describe alternate states in some smaller unit. For example, a muton consisting of the base pair A:T may have T:A, G:C, and C:G as allelic states. The presence of other bases or of other chemical configurations at the site would yield more possibilities, and deletion or duplication of the site might also be recognized as additional allelic states.

The fine structure of genes has been revealed in all organisms where it has been looked for; apparently it is a general situation. The allo-genic transformations of capsule type in pneumococcus (Figure 5.12) and the transformations yielding double capsular types and intermediate levels of sulfonamide resistance are probably achieved through recombination within a functional unit. Genes concerned with the fermentation of lactose have been studied in *E. coli* heterozygotes. One, responsible for the permease that pumps lactose into the cell, is divisible into five mutable sites which, when mutant, revert at different rates. There are also closely linked cistrons concerned with the synthesis of the enzyme, lactase, and with the inducible versus constitutive nature of this enzyme. A series of linked cistrons affecting the utilization of

galactose has been analyzed by means of transduction heterogenotes produced by the use of phage λ . One affects the enzyme — galactokinase, which phosphorylates galactose. A second, divisible into at least four mutable sites, concerns the galactose phosphate-uridyl transferase which incorporates galactose phosphate in a uridine nucleotide. This is the same enzyme that is absent in cases of human galactosemia.

Clearly genetic techniques have made possible an astonishingly detailed analysis of the structure of the DNA molecule. It is far from exact and far from complete, but it exceeds anything yet attempted by chemical techniques, where the first formidable problem is the separation of the thousands of species of molecules that make up a DNA preparation. When such a high order of specificity exists, chemistry needs to be supplemented by biological techniques; in fact, genetics is creating a novel chemistry of its own. It could be described as splitting the gene. The high power of resolution of genetics can be indicated by the facts in Table 6.4.

In view of the estimates and assumptions that go into such calculations, judgment of the exactness of these figures should be suspended. So far, not even in phages can a reasonable estimate be made of the amount of DNA that is genetic. Furthermore, in the mouse and in corn, the minimum map distances measured would certainly be vastly exceeded if more offspring could be studied. It is the smallest distances which reveal the resolving power of genetics, and from them we can see that units as small as one millionth of the linkage map can be identified, measured, and, to some degree, characterized. Such units are at the level of individual nucleotides. This raises the following questions: What is the unit of replication? How large is the smallest sequence of nucleotides whose pre-existence is needed for its own duplication? In duplication of a single gene, the nucleotides do not arrange themselves at random into *any* possible sequence, but only into the one existing in the template. How many nucleotides are needed for a unit to take on this function? Clearly one pair of nucleotides does not determine its own formation even though what seems to be a difference of one pair is replicated in the DNA molecule.

BIBLIOGRAPHY

- Beadle, G. W., 1955. Gene structure and gene function — L. J. Stadler Memorial Lecture. University of Missouri, College of Agriculture, Agricultural Experiment Station, *Research Bulletin* 588, 67 pp. (Includes L. J. Stadler's last paper, 1954. The gene. *Science*, **120**:811–819.)

- Benzer, S., 1955. Fine structure of a genetic region in bacteriophage. *Proc. Natl. Acad. Sci. Wash.*, **41**:344-354.
- Benzer, S., 1959. On the topology of genetic fine structure. *Proc. Natl. Acad. Sci. Wash.*, **45**:1607-1620.
- Demerec, M., Z. Hartman, P. E. Hartman, T. Yura, J. S. Gots, H. Ozeki, and S. W. Glover, 1956. Genetic studies with bacteria. *Carnegie Inst. of Wash. Publ.* 612, 136 pp.
- Genetics Soc. Am., 1955. Symposium on pseudoalleles and the theory of the gene. *Am. Naturalist*, **89**:65-127. (Papers by Green, Lewis, Laughnan, Stormont, and Stephens, all of whom work with higher plants or animals.)
- Pontecorvo, G., 1958. *Trends in Genetic Analysis*. 145 pp. New York, Columbia University Press.

7 Chromosome duplication and genetic recombination

An important goal of modern genetics is the description of events at the molecular level to account for the recombination of linked genes. The partial chiasmatype theory of crossing over, discussed in Chapter 4, provided a formal model of chromatid exchange at the four-strand stage, in keeping with the principal observations of classical cytogenetics. With the advent of microbial genetics, it became feasible to study recombination at a level of resolution not possible previously. The fine-structure mapping which resulted led to findings at variance with some of the basic tenets of the classical theory.

Individual deviations from the partial chiasmatype theory were first observed in *Drosophila* and subsequently in *Neurospora*, but for a time it seemed that only phage recombination operated on a different principle. Subsequently, more and more of the presumed special features of the phage system were regularly observed in *Neurospora* and in other organisms with a conventional meiosis. Recombination was shown to occur within a functional genetic unit, negative interference was demonstrated within very short map intervals, and aberrant segregation ratios of alleles were observed at meiosis.

These findings have compelled a revision of classical concepts of recombination. A detailed analysis of the molecular events underlying chromosome duplication and exchange at meiosis is needed for their understanding. Such an analysis is just beginning; the models of recombination thus far proposed are based primarily upon the available genetic data because there is not yet sufficient information available from chromosome studies. Nonetheless, questions of chromosome organization and duplication are under active investigation, and, because the results are of central importance for genetic analysis, we shall review

the present status of knowledge in this field. At the end of the chapter we shall return to the analysis of genetic recombination.

COMPARATIVE ORGANIZATION OF GENETIC MATERIAL

Studies of meiosis and genetic recombination in a great variety of sexual organisms from algae, fungi, and yeast to insects, higher plants, and mammals have underscored the fundamental uniformity of the process. Findings established in studies with one species have wide applicability to others. In contrast, processes of recombination in viruses and bacteria have some common features with the sexual ones, and some important differences. Because of the differences, it is important to distinguish carefully between genetic observations made in sexual and in other systems. The similarities and differences which we observe as particular patterns of recombination are the expression of specific structural features. Before proceeding to the details, a brief comparison of bacteria and higher forms with respect to the organization and duplication of the genetic material may be helpful.

The Nucleus

The nucleus of sexual organisms is a highly developed organelle, as seen in Plate IX, containing not only the chromosomes but also a special RNA-containing body, the nucleolus, which has not been found in bacteria. Also, the nucleus is surrounded by a porous double envelope, studded with RNA-containing granules on its outer cytoplasmic surface. No evidence has been found in electron micrographs of bacteria of any membrane between the DNA and the surrounding protoplasm (Plate II).

Organization of the DNA

The DNA of sexual organisms occurs in chromosomes which are complex nucleoprotein rods, consisting of DNA bound to a histone-type protein, and of other proteins and RNA. In bacteria, the DNA seems to exist in a relatively free form, both in uninfected and in phage-infected bacteria. In viruses, too, the nucleic acids seem to be free of specific attachment to the protein coats which surround them.

In sexual organisms, the interphase chromosomes exist as fibers below the resolving power of the light microscope, as does the DNA of bacteria.

Both can be visualized with DNA-specific staining reactions, as diffusely staining regions. In preparation for mitosis and meiosis, however, the interphase chromosomes condense to form readily visible structures, each with its own characteristic morphology. No analogous condensation has been seen in bacterial cells.

Replication of DNA

Recent studies have shown that DNA is replicated in the same manner in bacteria and in higher forms (p. 186). However, chromosome duplication involves synthesis and integration of other constituents besides DNA. The fact that DNA is synthesized almost continuously in multiplying bacteria, but occurs only at a specified and restricted time in cell division in higher forms, may be related to chromosome complexity.

Segregation of genetic material

Mitosis and meiosis represent the orderly systems by which sets of chromosomes are distributed to daughter cells after chromosome duplication has occurred. No comparable systems have been observed in bacteria, although it seems certain that some effective assortment process is at work, to insure the phenotypic uniformity of clones and maintenance of linkage relations that are observed. The special features of mitosis and meiosis, such as spindle formation, may be related to the problems presented by the presence of more than one chromosome per haploid set.

Recombination

Both sexual and other systems share a fundamental feature: the maintenance of the linear order of the genes, as evidenced by the constancy of linkage relations observed in mapping. To what extent the mechanism of recombination of linked genes is the same in all systems is a problem which has not yet been fully clarified.

How are these outstanding differences in structural organization and integration of the genetic material expressed in genetic behavior? Or, rephrasing the question, how do bacteria differ from higher forms at the level of cell heredity? There are no simple answers to these questions. In this chapter, we shall limit our considerations to DNA, chromosomes, and mechanisms of recombination, leaving questions of DNA-RNA interactions and the role of the organized nucleus for later chapters.

TABLE 7.1
DNA Content of Various Cell Types

	Values Given in Picograms = 10 ⁻¹² Grams Per Nucleus	
	Haploid	Diploid
Bacteriophage T4	2 × 10 ⁻⁴	
<i>E. coli</i>	1 × 10 ⁻²	
Yeast	2.3 × 10 ⁻²	
<i>Neurospora</i>	4 × 10 ⁻²	
<i>Aspergillus</i>	4 × 10 ⁻²	
<i>Drosophila</i>		1.7 × 10 ⁻¹
<i>Zea mays</i>		7
Mouse		5
Rat		6.5-7.6
Beef cattle	3.3	6.4-6.8
Man		6.0-6.8
Domestic fowl	1.3	2.4-2.6
Shad	0.91	1.99
Carp	1.6	3.0-3.3
Rainbow trout	2.45	4.9
Toad	3.7	7.3
Frog		15.0

CHEMICAL COMPOSITION OF CHROMOSOMES

Information concerning chromosome chemistry has been obtained with a variety of methods including staining with specific dyes, photometric measurements of chromosome-dye complexes and of UV absorption by unstained material, enzymatic hydrolysis, autoradiography to measure the incorporation of radioactive elements, and chemical studies of extracted nuclear constituents.

The localization of DNA in the chromosomes was first demonstrated by Feulgen in 1924 by the utilization of the Schiff reaction (mild acid hydrolysis to liberate purines from DNA, leading to aldehyde formation at the C-1 of the deoxyribose, and the appearance of a purple color from the reaction of aldehydes with basic fuchsin). DNA occurs elsewhere in cells in detectable amounts only under special conditions such as virus infection, and as storage material in the egg cells of some organisms. It has also been found in the basal bodies (kinetoplasts) of flagella.

Quantitative measurements of the DNA content per cell have been carried out by chemical extraction, which provides average values in

actual weight of DNA per cell, and by cytophotometric methods which provide comparative data in arbitrary units for individual cells. The methods complement one another to some extent.

Table 7.1 summarizes values obtained by a number of investigators for DNA content per cell in various organisms. There is a trend towards a greater amount of DNA per cell as organisms become larger and more complex, but no significant increase is found in organisms from fish to man. Table 7.1 also lists values for haploid sperm as well as for diploid cells of various tissues; in general, haploid cells have half the DNA content of diploids, and the diploid content is constant from tissue to tissue in each species. Variations which have been reported are too small to allow a definitive judgment of whether or not they are artifacts of the method.

In what physical state does the DNA exist in chromosomes? The best answer to this question has come from X-ray diffraction studies by Wilkins and co-workers of intact, isolated nuclei and of sperm heads, comparing the results with those from isolated DNAs. The evidence obtained supports the view that the majority, if not all, of the DNA is in the same helical configuration in the chromosome as it is in solution. However, as shown both by X-ray diffraction and by chemical procedures, cellular DNA forms a complex with a particular class of basic proteins—the histones—in all cells except bacteria and certain sperm; in the sperm, a simpler class of proteins—the protamines—have been substituted for histones.

Recent studies of the DNA-histone complex, DNP, extracted from calf thymus by exceedingly mild methods, indicate that the DNP is an elongate fibrous particle of about 18 million molecular weight, of which 8 million is attributable to a single DNA molecule, the rest to many histone molecules associated with the DNA in a stable configuration. It appears probable that at least half of the histone is in a helical configuration, and that both the DNA and protein extend the entire length of the fiber. Electron micrographs of these fibers shown in Plate III indicate a diameter of about 30 Å for the nucleoprotein, and about 20 Å for the DNA itself extracted from the DNP. Several investigators have proposed that the histones are wound around the DNA double helix, lying in the “deep groove” (Figure 1.2) where they can be held by the combined effect of many hydrogen bonds and van der Waal electrostatic forces. There is as yet no critical evidence that this interpretation is correct, but a number of observations, particularly from X-ray diffraction studies, do support it.

Mirsky and Ris estimated that the chromosomes of calf thymus contain about 39 per cent DNA, a similar amount of histone protein, and

about 9 per cent of a nonhistone, tryptophane-containing protein which remains insoluble after extracting much of the DNA and histone, and which they called residual protein. They considered it the backbone protein to which the DNA and histone attach. Other work, especially with enzymatic hydrolysis, supports the view that neither the DNA nor the histone are essential constituents of the backbone, because the removal of either one does not disintegrate the structure. However, some investigators view the chromosome as a complex with no single constituent comprising the framework for the others.

Chromosomes also contain RNA, but its content per nucleus has been found to vary greatly in cells of different tissues and from one organism to another. Values which have been reported include both chromosomal and nonchromosomal RNAs, because as yet no satisfactory techniques have been developed for isolation of the various nuclear components. Information about chromosomal RNA has come primarily from cytochemical studies, which have demonstrated cyclical fluctuations in the amounts of RNA bound to the chromosome. There is no definite evidence whether any RNA is a permanent chromosomal constituent. (See Chapter 11 for further discussion.)

Generally, chromosomes are visible only in the division stages of mitosis and meiosis. During interphase the nucleus appears optically homogeneous. However, the presence of DNA in these nuclei has been demonstrated by UV microscopy, by staining, and by chemical extraction. Why cannot the chromosomes of interphase nuclei readily be seen with the light microscope? As chromosomes become visible in early prophase, they appear as long, thin fibers; metaphase chromosomes are short, thick rods. The transition is achieved by coiling. In large meiotic chromosomes, such as those of the plant *Tradescantia*, one can see two orders of gyres, and there the mechanism of chromosome condensation by coiling is apparent. It is tempting to assume that submicroscopic coiling occurs too, and that the visible chromosome may be simply the condensed product of many ranks of coils, sufficient to bring the chromosome from a diameter of less than 100 Å to that of a metaphase chromosome 1000 times thicker.

ELECTRON MICROSCOPE STUDIES OF CHROMOSOMES

Studies with the electron microscope are just beginning to fill the gap between light microscope observations and physical-chemical studies of the molecular composition of the chromosome. Observations are not yet adequate to provide an understanding of chromosome structure.

A brief summary of the principal current hypotheses will indicate the present state of the knowledge.

1. The multistrand hypothesis proposes that the chromosome is a bundle of fibrils, each about $100 \text{ \AA}$ in diameter, and associated in pairs of pairs, so that the interphase chromosome before duplication consists of 32 fibrils. The $100 \text{ \AA}$ unit itself, which has been seen in cross section by many investigators, is considered to consist of 2 or 4 DNA double helices with associated protein. X-ray diffraction studies of intact sperm heads and isolated nuclei have indicated the presence of a nucleoprotein unit some $20 \text{ \AA}$ in diameter. How many of these units are present in the $100 \text{ \AA}$ fibril is not established experimentally as yet.

Doubleness of strands has been reported by cytologists at all levels between the $100 \text{ \AA}$ fibril and the prophase chromosome, but some of these observations may represent chance associations.

Support for the multistrand hypothesis comes not only from cytological observations but also from the logistic problem of packing tremendous lengths of DNA into short, fat chromosomes. Objections come first from genetic evidence, in which the segregation of new mutations and the 1:1 segregation of alleles are difficult to explain on a multistrand hypothesis, and from experiments with tritium-labeling of chromosomes (p. 191) in which the segregation of DNA subunits can be most simply visualized in terms of a duplex rather than a multiplex structure.

2. According to the single-strand hypothesis, the chromosome consists of a linear array of DNA molecules (about 10^3 in bacteria; as many as 10^5 in some chromosomes) combined with various proteins. When fully extended, it is at most $100 \text{ \AA}$ in diameter, and millimeters or even centimeters in length. It is capable of tremendous contraction by coiling or folding.

Cytological evidence in support of a single-strand hypothesis comes from correlated light and electron microscope studies of "lampbrush" chromosomes, found in meiotic prophase in amphibian eggs. These giant chromosomes, which attain lengths of more than a millimeter, consist of a central axis, from which pairs of loops project at right angles. The central axis consists of a row of granules (chromomeres) held together by a fine strand. This axis is Feulgen-positive, readily dispersed by the enzyme DNA-ase, but relatively resistant to dilute saline, ribonuclease, and the proteolytic enzymes, pepsin and trypsin. The loops also contain DNA and are heavily coated with RNA and proteins, which can be removed enzymatically, leaving a delicate core less than $500 \text{ \AA}$ in diameter. Figure 7.1 shows a drawing of a section of lampbrush chromosome as seen with the light microscope, together with a schematic

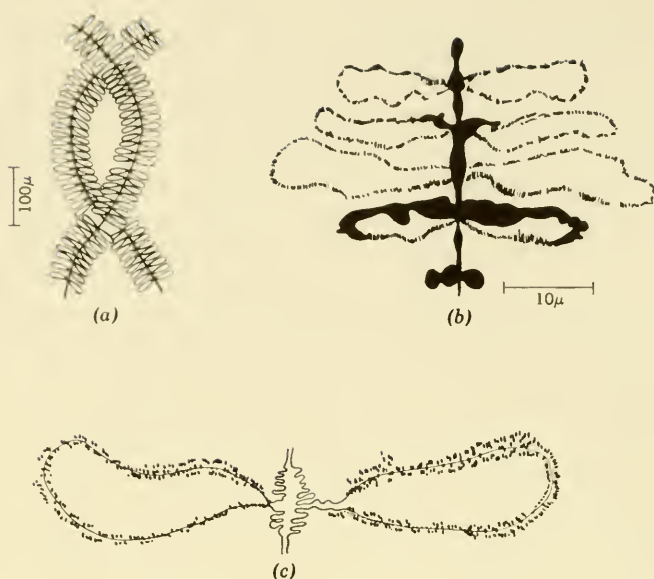


FIGURE 7.1. Lampbrush chromosomes of amphibian oöcytes (from J. G. Gall, 1958, in: *The Chemical Basis of Development*, Baltimore, The Johns Hopkins Press, pp. 103–135).

(a). A very diagrammatic representation of a lampbrush chromosome showing the homologues held together at two chiasmata. Hundreds of loop pairs extend laterally from the axis of each homologous strand.

(b). A somewhat schematic view of a lampbrush chromosome to show the central axis of the chromomere, a very delicate strand (usually invisible with the light microscope). Loop pairs of differing morphology project laterally from the axis. The loops are more closely spaced on the actual chromosome.

(c). Diagram of the postulated structure of a lampbrush chromosome based upon electron microscope studies and enzyme digestion.

diagram interpreting its structure on the basis of enzymatic and electron microscope investigations. The chromomeres are postulated to be highly condensed coils of the pair of chromosomes which make up the double continuous fiber running through both the axis and the loops, one chromosome on each side. (The homologous chromosomes are paired, this being meiotic prophase.) Measured at its narrowest point, in the fine strand between chromomeres, the chromosome is only 100–200 Å in diameter.

Most chromosomes do not provide material for structural analysis comparable with the lampbrush chromosomes of amphibian oöcytes. None-

theless, it is possible that a single-stranded nucleoprotein backbone, similar to that of the lampbrush chromosome, but coiled and contorted in specific regions, may represent the common basis of chromosome organization. Recent attempts to correlate chromosome structure with genetic and autoradiographic findings have taken into account only the number of strands required by the data. This turns out to be one DNA double helix per interphase chromosome before duplication. On this scheme, what cytogeneticists call the half-chromatid is equivalent to a single DNA strand. We shall reconsider chromosome structure at the end of this chapter.

INTEGRATION OF DNA IN THE BACTERIAL CELL

Studies of the macromolecular architecture of the bacterial cells are mostly very recent, and as yet no definitive picture has emerged. Here we shall present a current view which may change as more data become available.

Bacterial DNA is seen under the light microscope as Feulgen-positive material. The region where it is found corresponds to an area, as seen in sectioned cells in the electron microscope (Plate II), of very low electron density, containing or consisting of fine fibers 30–60 Å in diameter, probably highly hydrated. There is an abrupt transition between this area and the surrounding dense cytoplasm, but no evidence has been obtained of a nuclear membrane. Cells have been observed at all stages of division, but no apparent differences have been noted in the physical state of the DNA which might correspond to the condensation and coiling of chromosomes in higher organisms.

In fact, nothing comparable to mitosis has been seen in bacteria, in the opinion of most competent cytologists.

Since it has been established that the DNA forms a complex with histones in most cells of nuclear organisms and with protamines in some sperm, it was of particular interest to determine whether bacterial DNA also existed as a nucleoprotein complex. In a recent study, DNA was extracted from *E. coli* under extremely mild conditions in an attempt not to dissociate any existing DNA-protein complex. The extracted DNA was found in association with a nonhistone protein. X-ray diffraction studies of the complex indicated that at least half of the DNA was not bound to the protein, and that a large part of the protein was not bound to the DNA.

The striking common feature of all these systems is their fibrillar organization: whether DNA or nucleoprotein, the material consists of

fibers of molecular dimensions, 100–200 Å in diameter for chromosomes, 30 Å for extracted DNA-histone, 20 Å for DNA fibers alone.

Let us turn now to a consideration of replication and duplication of these genetic materials, processes which occur when the chromosomes are uncoiled.

DNA AND CHROMOSOME DUPLICATION

Here we shall distinguish between *duplication* and *replication*. Duplication is a general term, referring to any process by which a single structure becomes two identical ones. The structure in question may be a molecule, an aggregation of molecules, an organelle, a cell, or an organism; and the mechanisms involved may be equally diverse. Replication, on the other hand, refers to a unique process in which a molecule or aggregate is duplicated, with full conservation of specificity, by copying from a pre-existing molecule of the same kind. The only materials within the cell which are replicated are the hereditary materials; and, conversely, the demonstration that a particular material is replicated represents evidence that it is hereditary material. (For further discussion see Chapter 9).

We shall refer to the doubling of DNA as replication in view of the evidence to be presented below that it does replicate, as well as the inference from its role as hereditary material that it should do so. Regarding chromosomes as a whole, however, we can speak only of duplication, since the processes by which the non-DNA components are synthesized and assembled are not yet known.

Time of Chromosome Duplication

Although cytologists studied the chromosomes of mitosis and meiosis very systematically, they learned little about the duplication process for the excellent reason, unknown until recently, that it occurs during interphase when the chromosomes cannot be seen with the light microscope. Recent cytochemical measurements of DNA per nucleus in cells at recognizable division stages of mitosis and meiosis have shown that the DNA content doubles during interphase. Nuclei in prophase and metaphase stages have the double amount, which is reduced back to the basal level by chromatid separation (Figure 7.2).

In the analysis of genetic recombination, the time of chromosome pairing in relation to the time of DNA replication is an important question. Clearly, homologous chromosomes must be paired for exchanges to

occur. Cytochemical studies for the most part indicate that DNA replication is completed before chromosome pairing takes place; if so, recombination would necessarily be a consequence of breakage and exchange (chiasmatype theory), and not of events occurring during replication (copy-choice). A recent proposal, that recombination results from localized transient pairing during replication which is undetectable cytologically, followed by conventional pachytene pairing later, avoids the timing difficulty. This possibility will be discussed below (p. 202).

In healthy, rapidly multiplying bacterial cultures, DNA is synthesized continuously (or almost so) as demonstrated by the uniform rate of uptake and incorporation of radioactive thymidine into DNA by all the

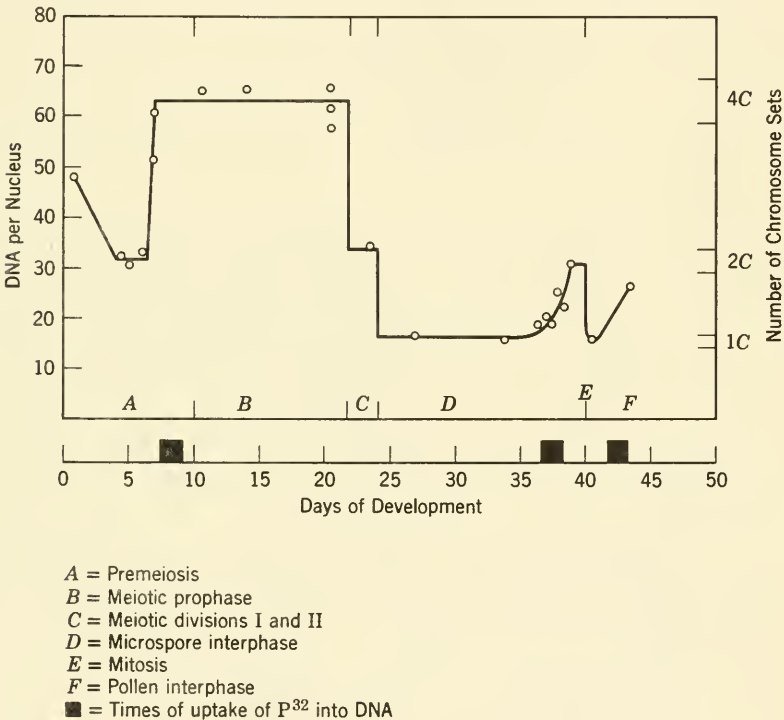


FIGURE 7.2. Relative DNA content of nuclei during meiosis and mitosis. In the anther of the lily, *Lilium longiflorum*, the DNA content per nucleus was determined photometrically by Feulgen staining. Anthers exposed to P³² as K₂P³²O₄ incorporated radioactivity into their DNA only at the specific times when chromosome duplication occurred, as shown by grain counts of autoradiographs correlated with photometric determinations of DNA content and with stage of development (after Taylor and McMaster, 1954, *Chromosoma*, 6:489).

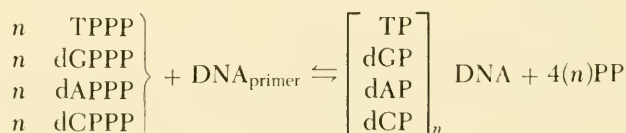
cells of a culture. The difference between bacteria, on the one hand, and plant and animal cells, on the other, in timing of DNA replication is striking, and probably reflects the difference in structural integration of DNA in the two systems. DNA replication itself is most likely an identical process in bacteria and in other organisms, as has been shown beautifully in the following experiments.

The replication of DNA

DNA in solution exists in the form of double helices, pairs of polynucleotide chains coiled around each other in a precise manner such that hydrogen-bonding is possible between adjacent nitrogen bases of the two strands (Figure 1.2). The pairing is selective, adenine with thymine and guanine with cytosine. Thus the nucleotide sequence of either strand specifies precisely the sequence of the other strand which is complementary to it. This situation provides a formal basis for a template mechanism of replication, in which the two strands come apart, each determining the nucleotide sequence in a newly forming complementary strand (Figure 7.3).

In the few years since this replication model for DNA was proposed, a number of lines of evidence have been obtained which provide strong support for it. The enzymatic mechanism of DNA synthesis has been discovered and shown to require DNA as a primer or template. By the use of isotopically labeled DNA to distinguish between old and newly formed strands, the process of DNA replication has been followed in a number of organisms from bacteria to higher plants. Virtually identical results have been obtained with the use of entirely different methods, one employing DNA alone and the other autoradiographs of intact chromosomes.

In 1956, Kornberg and co-workers described an enzyme preparation from *E. coli* which was capable of synthesizing DNA. Since that time, intensive work with the system has clarified many of its features. The over-all reaction is:



The substrates for the reaction are the four deoxynucleoside triphosphates of adenine, guanine, cytosine, and thymine. All four are required for any detectable synthesis to occur, and a further requirement is the

presence of a small amount of DNA which serves as primer of the reaction. Each triphosphate is incorporated into the polynucleotide with the splitting off of pyrophosphate which is found in the reaction mixture in stoichiometric quantities. The reaction can be reversed when pyrophosphate is present in excess.

The DNA produced in this reaction has essentially the same physical properties as DNA carefully extracted from biological materials, with respect to molecular weight, viscosity, degradation by DNA-ase, and effects of heating. Of particular interest is the relation between the

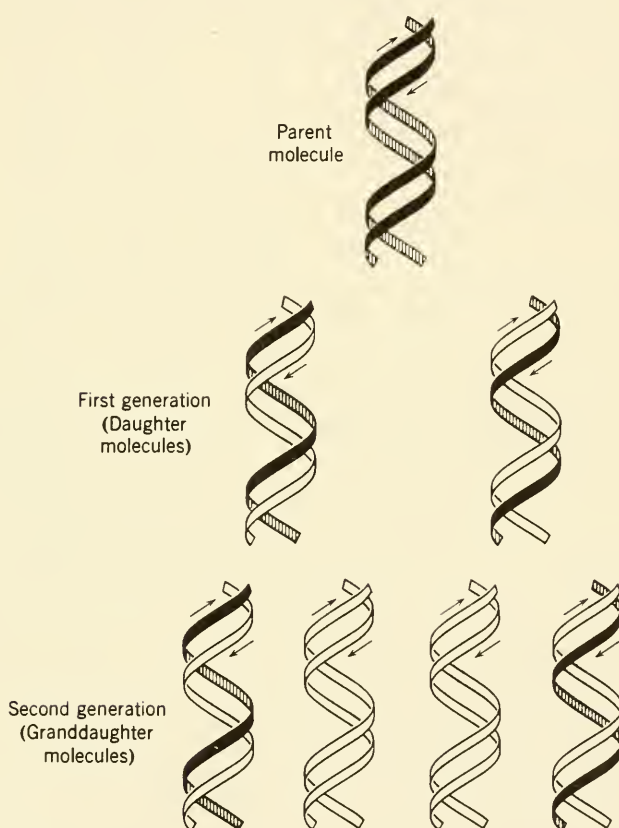


FIGURE 7.3. Proposed model of DNA replication. Parental strands of the DNA double helix are shown in black, and newly formed complementary strands are shown in outline. Arrows indicate direction of the strands in the sense that phosphate diester bonds connect the C-3 carbon of one sugar with the C-5 of the next. Complementary strands run in opposite directions (from Meselson and Stahl, 1958, *C.S.H. Symp. Quant. Biol.*, 23:10).

TABLE 7.2
Chemical Composition of Enzymatically Synthesized DNA,
Synthesized with Different Primers. A, Adenine;
T, Thymine; G, Guanine; C, Cytosine
 (From Kornberg, 1960, *Science*, **131**:1507)

DNA	A	T	G	C	$\frac{A + G}{T + C}$	$\frac{A + T}{G + C}$
<i>Mycobacterium phlei</i>						
Primer	0.65	0.66	1.35	1.34	1.01	0.49
Product	0.66	0.65	1.34	1.37	0.99	0.48
<i>E. coli</i>						
Primer	1.00	0.97	0.98	1.05	0.98	0.97
Product	1.04	1.00	0.97	0.98	1.01	1.02
Calf thymus						
Primer	1.14	1.05	0.90	0.85	1.05	1.25
Product	1.12	1.08	0.85	0.85	1.02	1.29
Bacteriophage T2						
Primer	1.31	1.32	0.67	0.70	0.98	1.92
Product	1.33	1.29	0.69	0.70	1.02	1.90
A-T copolymer	1.99	1.93	<0.05	<0.05	1.03	40

primer DNA employed and the new DNA synthesized. As can be seen from the examples cited in Table 7.2, there is a very close relation in base ratio between primer and product, strongly indicating that the primer serves as a template for an enzymatic replication of DNA.

It seems very likely that this enzymatic reaction represents the principal, perhaps the only, mechanism of DNA synthesis in the cell. The discovery of this system, consequently, represents a contribution of unique importance in the analysis of cell heredity.

Evidence that DNA is replicated by a mechanism which involves the laying down of a new polynucleotide strand upon a template consisting of a pre-existing DNA strand, comes also from an entirely different kind of experiment. A method was developed by Meselson and Stahl to distinguish between old and newly formed DNA by means of isotopic labeling. These experiments utilize the density difference between DNA molecules containing ordinary N^{14} and those in which the heavier isotope N^{15} has been substituted by growing cells with $N^{15}H_4Cl$ as sole nitrogen source.

It has recently been established that N^{14} - and N^{15} -containing DNA molecules can be well separated by equilibrium centrifugation in a

solvent of appropriate density, approximately 7M cesium chloride in water. The small molecules of cesium chloride are distributed across the cell of the ultracentrifuge in a continuous density gradient, but the huge DNA molecules collect in a narrow band corresponding to their density. Molecules of uniform density are distributed in the region of the band as a result of thermal agitation, and from the band width the molecular weight can be computed. If there is a distribution of density differences among molecules of a particular sample, there will be a further broadening of the band. If the molecular weight and size homogeneity of the sample are known from other experiments, then the bandwidth can be used to compute density differences within the sample. This method is so sensitive that N^{14} DNA and N^{15} DNA separate readily into distinct bands.

The experiment consists of growing bacteria (*E. coli*) with an N^{15} -containing nitrogen source until the DNA is well labeled, then allowing any desired amount of growth with an N^{14} nitrogen source, and finally observing the pattern of DNA distribution in the ultracentrifuge.

The results are remarkably clear cut, as can be seen in Figure 7.4. Initially only one band is found, corresponding to N^{15} DNA. After one doubling in the presence of N^{14} , again one band is found, but in a new position. It is intermediate between N^{15} DNA and N^{14} DNA. After a second doubling, two bands are found of equal DNA concentration. One is the intermediate band, and the other is still less dense, and is in the position of N^{14} DNA. Subsequently, the intermediate band decreases by half after each doubling and the N^{14} DNA band increases correspondingly. Similar results have been obtained with *Chlamydomonas* and with mammalian cells in tissue culture.

The intermediate band contains equal amounts of N^{14} and N^{15} DNA, combined in such a way that neither the salt concentration nor the centrifugal forces dissociate them. However, by heating under conditions that break hydrogen bonds, the mixture has been dissociated into two subunits, one containing all N^{14} and the other all N^{15} , and each with a molecular weight half that of the original molecule. It may be inferred, therefore, that the band appearing after one doubling contains hybrid molecules, each consisting half of an N^{15} subunit and half of an N^{14} subunit. It is probable that the subunits are the two strands of the double helix (although more complicated models can be devised to account for the data).

On the hypothesis that the subunits are individual polynucleotide strands, the interpretation of the experimental results is straightforward, as diagrammed in Figure 7.3. In the first replication, each N^{15} strand

Exp. no.

Generations

1

0

1

0.3

1

0.7

2

1.0

1

1.1

1

1.5

1

1.9

2

2.5

2

3.0

2

4.1

1

0 and 1.9
mixed

2

0 and 4.1
mixed

(a)

(b)

is paired with a complementary new strand composed of N^{14} nucleotides from the medium. Thus all the replicated DNA is hybrid. At the next replication, all the new strands are N^{14} , but so are half of the old strands. At subsequent replications, each hybrid molecule gives rise to one hybrid and one N^{14} molecule. The original N^{15} strands maintain their integrity and have been followed experimentally for four replications.

The principal findings established by this experiment are: (1) DNA consists of bipartite molecules, each containing two subunits; (2) after each replication, the progeny DNA molecules are again bipartite, consisting of one old and one new strand; and (3) each DNA subunit retains its structural integrity for at least four replications and perhaps indefinitely. DNA replication, then, consists of the doubling of duplex molecules to produce two duplexes, each consisting of one old and one new monoplex strand.

What is the relation of the organized DNA of the intact cell to the DNA molecules which, when extracted from *E. coli*, sediment in the ultracentrifuge? *E. coli* contains about 6×10^9 molecular weight of DNA per bacterial nucleus; the extracted DNA is quite homogeneous in size, with a molecular weight of about 7 to 8×10^6 as determined by the cesium chloride method. On this basis, the extracted DNA consists presumably of about 1000 pieces. The genetic evidence based upon mapping of mutants indicates the presence of only one linkage group or chromosome in *E. coli*, and therefore it seems likely that within the cell the DNA is organized into a single structure and that it fragments upon extraction.

Chromosome duplication

DNA replication has also been studied in a number of plants, by methods in which the unit of observation was not the DNA molecule

FIGURE 7.4. Ultraviolet absorption photographs showing DNA bands resulting from density-gradient centrifugation of lysates of bacteria sampled at various times after addition of excess N^{14} substrates to a growing cultures of N^{15} -labeled cells. Regions of equal density occupy the same horizontal position on each photograph. The generation times were estimated from measurements of bacterial growth. The densitometer tracings shown in (b) are proportional to the DNA concentrations. The degree of labeling of a species of DNA corresponds to the relative position of its band between the bands of fully labeled and unlabeled DNA shown in the lowermost frame, which serves as a density reference. A test of the conclusion that the DNA in the band of intermediate density is just half-labeled is provided by the frame showing the mixture of generations 0 and 1.9. The peak of intermediate density is centered at 50 ± 2 per cent of the distance between the N^{14} and N^{15} peaks (from Meselson and Stahl, 1958, *Proc. Natl. Acad. Sci., Wash.*, 44:671).

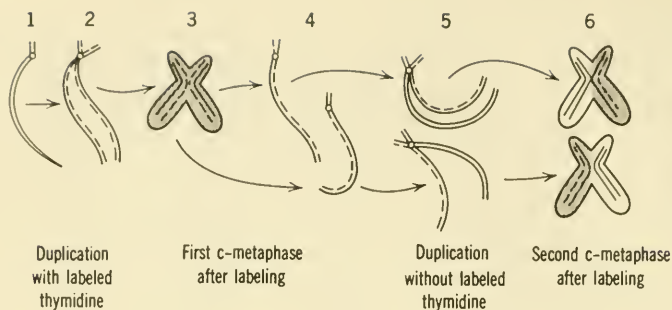


FIGURE 7.5. Chromosome duplication experiment (after Taylor, Woods, and Hughes, 1957, *Proc. Natl. Acad. Sci. Wash.*, **43**:122).

1. Chromosome before duplication; seen as one strand under the microscope, it is revealed to be bipartite in this experiment.

2. Chromosome after duplication in presence of radioactive thymidine- H^3 .

3. Appearance of metaphase chromosomes, showing uniform labeling.

4. Separation of the uniformly labeled chromosomes.

5. Duplication in the absence of radioactive thymidine- H^3 .

6. Duplicated chromosomes at the next metaphase: one chromosome is radioactive, the other is not.

Diagrams 2, 4, and 5 provide interpretation of observations shown in 3 and 6. Uniform labeling at the first metaphase but not at the second metaphase results from the bipartite nature of the chromosomal DNA. Solid lines indicate unlabeled strands; dotted lines indicate radioactive strands; shadowing indicates grains seen in the autoradiographs, resulting from the presence of thymidine- H^3 .

but the DNA-containing chromosome. These experiments by J. H. Taylor are formally similar to the N^{15} experiments in that isotope labeling of the DNA was used to distinguish old and new strands.

Chromosomes were labeled with radioactive thymidine, a nucleoside that goes only into DNA. The radioactivity came from tritium (radiohydrogen H^3), substituted for hydrogen in a stable position where it does not exchange appreciably with hydrogen in the medium. Thus it effectively labels the location of incorporated thymidine, and consequently of DNA, in material from which all low molecular weight thymidine has been removed. To observe the location of the radioactive element, a photographic emulsion used to record disintegrations is placed tightly against cells or sections mounted on slides. After a suitable exposure time followed by development, the slides are examined together with the overlying emulsion under the microscope. It is possible to determine very accurately the location in the chromosome of the tritium, which is the source of electrons which reduce the silver grains of the emulsion following nuclear disintegration.

The experimental procedure consisted of soaking root tips in a high

concentration of tritiated thymidine for a time interval such that at most one cycle of DNA duplication had occurred in some cells. Then the material was mounted and autoradiographs were prepared. As shown in Figure 7.5, uniform labeling of the two daughter chromosomes was observed after one cycle of duplication. In the next experiment, cells were allowed to undergo two successive divisions, one in the presence of labeled thymidine and one in its absence, and the progeny chromosomes were kept together by the use of colchicine. At the second division, segregation of labeled and unlabeled strands occurred, as diagrammed in Figure 7.5. When chromosomes were allowed to undergo a third division before observation, one labeled strand was still found as well as seven unlabeled ones, representing the eight progeny of an individual chromosome labeled before its first division.

These results demonstrate that the chromosome is (at least) double, consisting of two DNA subunits, both of which are replicated and then segregated from one another at the first division, resulting in equal labeling of the daughter chromosomes which are themselves double. Their doubleness becomes apparent at the second division when the old unlabeled and new labeled subunits segregate from each other.

This experiment also shows that each subunit remains intact in the replication process, so that an old and a new subunit can be distinguished. This is extremely important, for it represents direct evidence that the linear structural organization of the many DNA molecules in the chromosome is kept intact during replication (except for sister-strand exchange; see below).

The N^{15} DNA experiment beautifully complements this one. Both systems provide evidence of the same mechanism of DNA replication: namely, the doubling of a duplex structure in such a way that two duplexes are produced, each containing one old and one new monoplex.

In the plant chromosome experiments, additional information was obtained about the organization of the DNA, because observations were made of DNA segregation in intact chromosomes. It was noted that the linear organization of the DNA does not always remain intact for the entire length of the chromosome. Occasionally a chromosome is labeled for only part of its length, and the sister chromosome is labeled reciprocally. In these instances, each pair of chromosomes has the equivalent of one labeled and one unlabeled strand, but reciprocal exchanges have occurred between sister half-chromatids. These exchanges may be a regular feature of chromosome replication, occurring in the plant *Bellavalia* with an average frequency of once per chromosome per replication. As yet nothing is known of the mechanism of the exchange, or even whether it is an essential concomitant of the replication process.

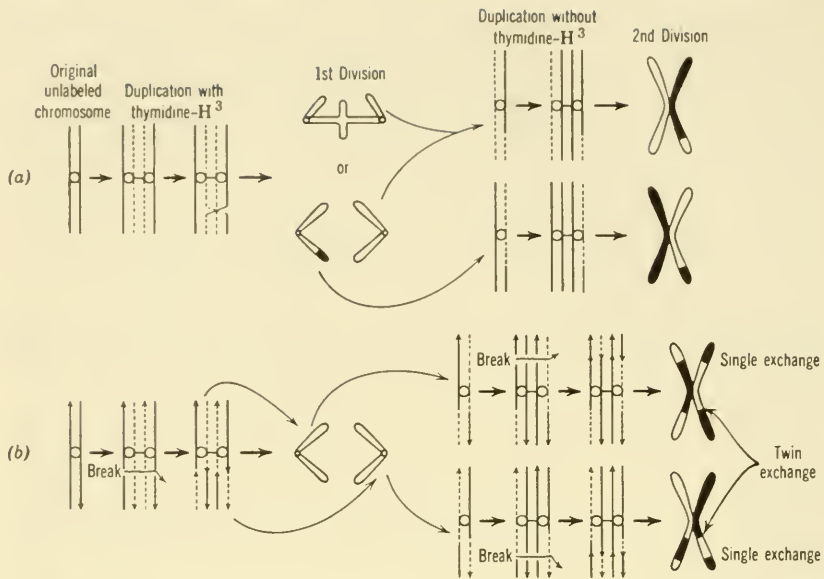


FIGURE 7.6. Interpretation of sister-chromatid exchanges found in studies of chromosome duplication with thymidine- H^3 (from Taylor, 1958, *Genetics*, 43:515).

(a). The type of chromosome configuration resulting from a half-chromatid break, namely, an unpaired segment with loss of label in one of the two daughter chromosomes, is not found.

(b). Reciprocal exchanges are found either in two (so-called single exchanges) or in four (twin exchanges) of the daughter chromatids after two duplications. The pattern of reciprocal exchanges found is evidence of polarity in the rejoining of broken ends after exchange, with half-chromatids (equivalent perhaps to a single DNA strand of the double helix) only rejoining with those of the same polarity.

These exchanges are also of interest because they permit a test to be made of whether or not the subunits are identical. If exchanges involved only two of the four strands, one new with one old, then patterns like that shown in Figure 7.6a should be found, but in fact they are not. From the labeling patterns which are found, it is concluded that exchanges involve all four strands, as shown in Figure 7.6b. One may then ask: Do exchanges among the four strands occur at random as would be expected if the strands were identical? If so, the expected frequencies of single and twin exchanges after the second replication would be about 10:1. But the ratio of frequencies actually found is about 2:1. How can this be explained?

The model which best fits these data is one which considers the pair of DNA subunits as complementary, not as identical strands, and limits

exchanges to those occurring between identical strands. The physical basis of complementarity could be the DNA double helix itself. The complementary strands run in opposite directions in the sense that the phosphate diester bonds connect the C-3 carbon of one sugar with the C-5 of the next, (Figure 1.1) and this alone may exclude end-to-end joining of complementary chains. Thus the data from sister-strand exchanges supports the hypothesis that the DNA subunits observed by tritium labeling actually correspond to the two strands of the DNA double helix.

Recently, DNA replication in intact chromosomes has been studied by exposing cells to thymidine- H^3 for very short intervals. In general, evidence has been obtained of asynchronous replication in plant (*Crepis* and rye) and in animal material (grasshopper and Chinese hamster). A detailed analysis of Chinese hamster cells in tissue culture has shown that several of the chromosomes have segments which are typically late in duplication. For example, the DNA of the long arm of the X chromosome is replicated in the last half of the interval of DNA synthesis, while that of the short arm is replicated during the first half of this period. Does the time of DNA replication coincide with the time of chromosome duplication? It has been shown that chromosomal histones are synthesized at approximately the same time as is DNA. Although the analysis has not been pursued with the precision of the DNA labeling experiments, it is likely that the asynchrony of DNA replication applies also to other components of the newly forming chromosome.

This information all comes from studies of mitosis. For the analysis of recombination mechanisms, one requires similar observations of chromosome duplication in meiosis, and such experiments are now being carried out. However, the mitotic chromosomes do provide some genetically relevant information: (1) that each chromatid contains two DNA subunits, which are probably the two strands of the double helix; (2) that in general the old subunits remain intact during replication of new strands, but occasionally sister-strand exchanges may occur; and (3) that exchanges involve recombination of all four strands, not at random, but limited to rejoining of like-directed chains.

RECOMBINATION REVISITED

Having surveyed the available knowledge about chromosome organization, we may now return to the central theme of this chapter: possible mechanisms of genetic recombination. The issue was reopened by the results of phage crosses which differed from those observed with higher

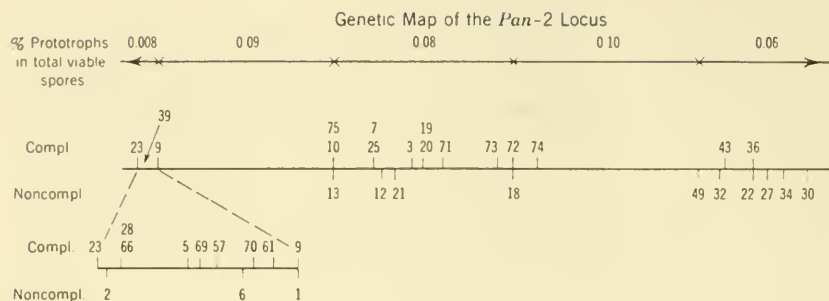


FIGURE 7.7. Mutants at the *pan-2* locus in *Neurospora* (after Case and Giles, 1960, *Proc. Natl. Acad. Sci. Wash.*, 46:659).

forms in every respect except one: the linearity of the genetic material. With the application of fine structure mapping to *Neurospora* and other microorganisms, instances were regularly found which were rather similar to the results obtained with phage. Let us summarize these observations, aberrant from the view point of the chiasmatype theory of crossing over (Chapter 4).

Recombination within a Gene

The appearance of wild-type progeny in crosses between two allelic mutants was described in Chapter 6. Alleles behaving in this way were initially called pseudoalleles, because it was not considered possible for recombination to occur between true alleles, which were considered indivisible. Pseudoalleles were first detected by E. B. Lewis in *Drosophila*, and pursued in this organism by him and by M. M. Green, in intensive studies, which were very laborious because of the low frequency of recombinants recovered. Most of the current studies are being carried out with microbial systems in which selective techniques permit the screening of many millions of offspring with ease.

Wild-type progeny have been recovered from crosses between allelic mutants at a number of different loci in *Neurospora*, yeast, and *Aspergillus*, as well as in phage. Do these exceptional progeny actually result from recombination? The strongest evidence for recombination is provided by the *rII* region of phage (Chapter 6) in which mutants have been mapped by recombination and also by deletion analysis virtually to the nucleotide level.

Similar results, but less detailed, have been obtained in *Neurospora*, for example, at the *pan-2* locus shown in Figure 7.7. All mutants at

this locus require pantothenic acid for growth, and are found in a small region of linkage Group VI, between the genes *ylo* and *tryp-2*. Some 40 mutant alleles were mapped in mutant $\times$ mutant crosses, on the basis of the frequency of wild-type progeny (assumed to be recombinants) as well as recombination with outside markers. The linearity of the resulting map provides strong evidence that these offspring did arise by recombination. Had they resulted from some other process, their frequency should not have been a function of distance along a linkage map.

In *Neurospora* and in yeast, most of the data on recombination between alleles come from enumeration of random ascospores. For example, the results listed in Table 7.3 are based upon plating hundreds of thousands of ascospores onto minimal medium, and counting the number of colonies which form. In a few systems, fortunately, tetrad analysis was also undertaken, and unexpected results of great importance were obtained. For example, over 1700 asci were dissected in crosses of alleles at the *pan-2* locus. Nineteen of these asci showed evidence of recombination within *pan-2*. In only two of these asci was the recombination event within the locus reciprocal, giving rise to the expected four types of progeny. These two asci provide evidence of reciprocal exchange within a locus comparable to that observed between loci in classical genetic analysis.

Aberrant Tetrads

The remaining 17 exceptional asci show nonreciprocal recombination within the *pan-2* region, and fall into three classes, as shown in Figure

TABLE 7.3
Wild-Type Recombinants Recovered in
Mutant $\times$ Mutant Crosses in *Neurospora*

Cross	% Wild Type	Total Number of Progeny Observed
<i>pdx</i>		
<i>pdx</i> $\times$ <i>pdxp</i>	0.18	62,777
<i>pdxq</i> $\times$ <i>pdxp</i>	0.12	37,232
<i>pdx</i> $\times$ <i>pdxq</i>	none	35,000
<i>pan-2</i>		
9 $\times$ 7	0.22	421,171
18 $\times$ 9	0.40	252,982
18 $\times$ 6	0.31	414,510
10 $\times$ 9	0.09	573,220
22 $\times$ 6	0.46	206,945

Parents $a+ \times +b$

(a and b are alleles at the same locus)

(A) Reciprocal recombination

$a+$
 ab
 $++$
 $+b$

(B) Nonreciprocal recombination

Class 1:	$a+$ $+b$ $++$ $++$	$a+$ $+b$ $+b$ $++$	$a+$ $a+$ $+b$ $++$
Class 2:	$a+$ $a+$ $++$ $++$		
Class 3:	$a+$ $a+$ $a+$ $+b$	$a+$ $+b$ $+b$ $+b$	

FIGURE 7.8. Aberrant tetrad types found in *Neurospora*.

7.8, which also includes data from a study of the *pdx* region of *Neurospora*. Class 1 includes asci with only one recombinant and both parental types; class 2 consists of asci with only one parental and one recombinant type; class 3 contains only parental types, segregating 3:1 instead of the expected 2:2.

Two mechanisms have been proposed to account for these data. One mechanism, called gene conversion, postulates some sort of directed mutation in which one allele influences the configuration of its homolog. This mechanism does not account for classes 1 and 2, in which new phenotypes are found unlike either parent. Rather than directed mutation, the other mechanism involves recombination by a copy-choice method, similar to that proposed for phage recombination. At the level of individual nucleotides, the distinction between directed mutation and copy-choice recombination is probably meaningless.

Negative Interference

Another new phenomenon associated with intragenic recombination is negative interference: the increased frequency of outside marker exchange in the vicinity of an intragenic recombination event over that occurring in unselected material. In *Neurospora* there is almost random

TABLE 7.4
 Reciprocal Crosses Between Noncomplementing Group B3 and Group B5 Mutants.
 (Mutants in Group B5—9, 6. Mutants in Groups B3—7, 10, 18, 22)
 (From Case and Giles, 1958, *C. S. H. Symp. Quant. Biol.*, 23:127)

Cross	No. of Viable Ascospores	No. of <i>pan</i> Prototrophs	Frequency of <i>pan</i> Prototrophs	% <i>pan</i> Prototrophs in the Four Classes ^a			
				Noncrossover		Crossover	
				p1	p2	+	+
9 <i>glo</i> × 7 <i>tryp</i>	164,920	351	0.21	28.0	21.0	34.0	25.0
7 <i>glo</i> × 9 <i>tryp</i>	256,251	597	0.23	34.0	29.0	14.0	22.0
18 <i>glo</i> × 9 <i>tryp</i>	105,710	380	0.35	27.0	25.0	11.0	37.0
9 <i>glo</i> × 18 <i>tryp</i>	147,272	645	0.44	22.6	17.5	47.4	12.3
18 <i>glo</i> × 6 <i>tryp</i>	163,800	554	0.33	27.0	22.0	13.0	37.0
6 <i>glo</i> × 18 <i>tryp</i>	250,710	710	0.28	26.0	26.0	33.0	14.0
10 <i>glo</i> × 9 <i>tryp</i>	285,250	266	0.09	29.5	34.7	9.3	26.4
9 <i>glo</i> × 10 <i>tryp</i>	288,970	270	0.09	22.0	32.3	26.1	19.4
22 <i>glo</i> × 6 <i>tryp</i>	56,445	360	0.63	6.0	26.0	11.0	57.0
6 <i>glo</i> × 22 <i>tryp</i>	150,500	420	0.28	22.6	22.6	45.4	10.0

^a Data based on a sample of 100 prototrophs tested from each cross.



FIGURE 7.9 (a). Segregation of a gene pair controlling light and dark spore wall color in ordered asci of the fungus *Sordaria*. Only 4:4 segregations are seen in this group. The gene-centromere map distance can be computed directly from the frequency of asci showing other than 4 light:4 dark spores in linear array.

(b). An aberrant ascus from the same cross as in a, with 6:2 segregation.

(c). An aberrant ascus from the same cross with 5:3 segregation (from L. S. Olive, unpublished).

assortment between outside markers among progeny showing recombination within a gene, and the total amount of recombination of linked markers is much higher in their exceptional progeny than in the general population. Some representative data are given in Table 7.4.

These results are clearly at variance with the massive evidence of positive interference observed between exchanges which are in the range of 1 to 10 map units apart. It may be recalled that when two loci are linked, the frequency of double exchanges between them is generally lower than that expected from the frequencies of single exchanges. The occurrence of one exchange appears to decrease the probability of a second exchange in the vicinity. This phenomenon is found in the yeasts and fungi as well as in higher forms. Any general theory of recombination must account for both positive and negative interference.

Aberrant Asci with 5:3 and 6:2 Ratios

Another phenomenon which must be accounted for is the occurrence of 5:3 and 6:2 segregation ratios of alleles. Although such ratios have been observed in *Neurospora*, the most extensive data come from studies by Olive with the related fungus, *Sordaria*. In this beautiful material, mutations occur which alter the pigmentation of the ascospore wall and, consequently, the segregation of alleles affecting spore color can be scored by direct observation of the undissected asci. A photograph of asci with 4:4 and 5:3 segregation ratios is shown in Figure 7.9. Some data on frequencies of various ascus types are given in Table 7.5.

M. Mitchell has performed a careful analysis of two unlinked loci in *Neurospora* to look for aberrant ratios of this type. She found that one locus showed 25 irregular segregations in 109 asci dissected, whereas the

TABLE 7.5
Aberrant Tetrads in *Sordaria*
(From Kitani, Olive, and El-Ani, unpublished^o)

Aberrant Ratios	Number of Asci	
6+ : 2g	39	
5+ : 3g	50	90 with excess +
7+ : 1g	1	
2+ : 6g	8	
3+ : 5g	12	20 with excess g
Total	110	

^oIn this cross, 0.12 per cent of the asci were aberrant for g/+ segregation.

second locus showed none, giving only 4:4 segregations. What the relationship may be between 5:3 ratios and the other types of aberrant tetrads discussed above is not at all clear at present. It does seem likely that the frequency of aberrant allelic ratios at some loci may be much higher than was ever suspected in the past, when many such examples were discarded as technical errors.

Re-Evaluation

The data of classical cytogenetics are fairly consistent with the chiasmatype theory of crossing over, which holds that genetic recombination is the consequence of reciprocal exchanges between nonsister chromatids occurring after chromosome duplication and pairing, when the four chromatids lie close together, constituting the bivalent of meiotic prophase. Clearly, the new genetic data from studies of recombination in very short intervals cannot be interpreted on this theory.

The great difference in findings between the two systems results from the size of the region under observation. In studying recombination between markers which are several map units apart, one overlooks the events which are detected by selecting recombinants in regions smaller than 0.1 map unit. Intragenic recombination at a particular locus is generally too rare an event to be found in an unselected sample of progeny. A comparison is given in Table 7.6 of the observations made in conventional and in fine-structure mapping.

In evaluating these findings, two alternative views have been proposed: (1) a unified hypothesis, that all recombination is of the same type, based upon a copy-choice mechanism which operates during DNA replication and chromosome duplication accompanied by sister-strand exchange; and (2) a two-mechanism hypothesis, that the aberrant events of recombination within loci occur by copy-choice at the time of DNA replication and, in addition, reciprocal exchanges occur between any two of the four non-sister chromatids.

Preliminary attempts to distinguish experimentally between these alternatives have been based on the view that, if the mechanisms are different, it should be possible to alter the frequency of one process independently of the other. Treatments with UV and with heat have indicated that the approach is a promising one, but no conclusive results have been obtained as yet.

In the absence of conclusive experimental data, we may consider these two hypotheses in the light of existing information. If all recombination is of the same type, there is a timing problem. Replication of DNA seems to be completed before zygotene pairing of homologous chromo-

TABLE 7.6
Comparison of Conventional and Fine-Structure Mapping

	Selected Intragenic Recombinants		
	Unselected Recombinants	Yeast and Fungi	Phage
Interval observed	usually more than 1% recombination	less than 1% recombination	less than 1% recombination
Recombination within a locus	no°	yes	yes
Interference	positive	negative	negative
Reciprocal recombination	yes	intragenic — no outside markers — yes	no —
Aberrant allelic ratios	no°	yes	—

°Too rare to find by this method.

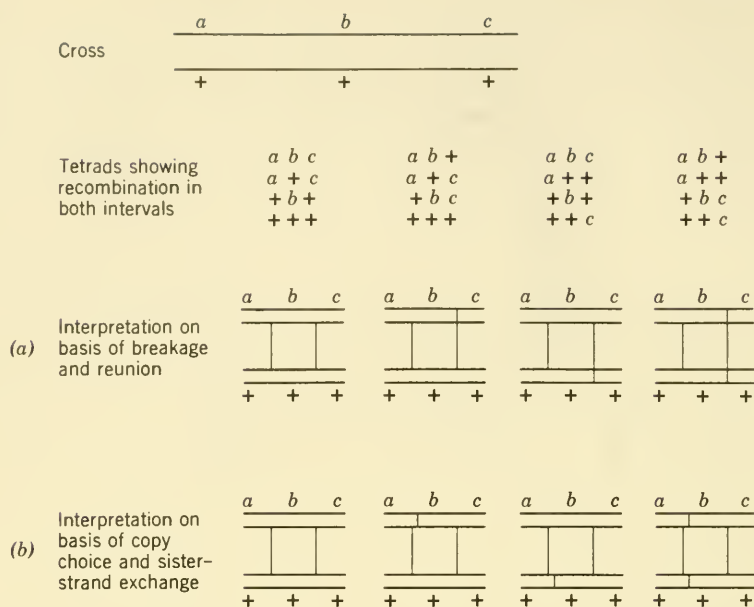


FIGURE 7.10. Genetic consequences of copy-choice recombination plus sister-strand exchange (from Pritchard, 1960, *Microbial Genetics*, Cambridge, The University Press).

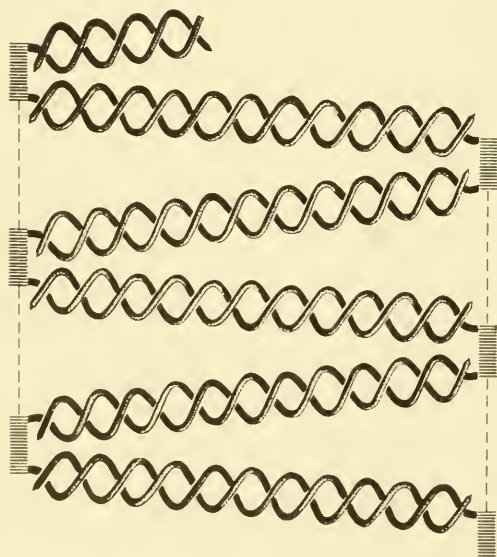


FIGURE 7.11. Diagrammatic representation of a linear model of a chromosome. The DNA particles (molecules?) are held together by links (protein?) which are assumed to provide great flexibility to the over-all structure, which can be stretched out, becoming virtually linear, or folded to form a ribbon. A genetic consequence is the possibility of two different kinds of exchange, one occurring at the link points, and the other occurring within the DNA helix, as for instance, in Figure 7.15 (after Freese, 1958, *Cold Spring Harbor Symp. Quant. Biol.*, 23:13).

somes begins. Yet the correlation of genetic crossing over with chiasma formation is good and supports the view that reciprocal exchanges do occur. One proposed escape from this dilemma is based on the idea of transient pairing within short, localized regions during DNA replication. On this scheme, recombination would be by copy-choice, with sister-strand exchanges also occurring, and complete pairing of homologous chromosomes taking place at meiotic prophase, as observed by cytologists. A model of this scheme is shown in Figure 7.10.

The two-mechanism hypothesis which at present appears more likely requires that the chromosome provide a suitable structural basis for both copy-choice and reciprocal exchange. A model of such a chromosome has been proposed by Freese and by Taylor, and adapted to the bacterial chromosome by Kellenberger. The essential feature of this model is that DNA molecules are held together by *links* in such a way that copy-choice recombination may occur between homologous DNA molecules during replication, and that reciprocal exchanges may occur at the links, then or later. A diagram of this model is shown in Figure 7.11, and a proposed scheme for nonreciprocal recombination within a locus is shown in Figure 7.12

In summary, then, this intriguing and fundamentally important area of recombination mechanics is in need of a far better understanding of the molecular organization of the chromosome than is now available. Genetic evidence does not exclude such diverse possibilities as: (1) that copy-choice is responsible only for rare events which mar the otherwise classi-

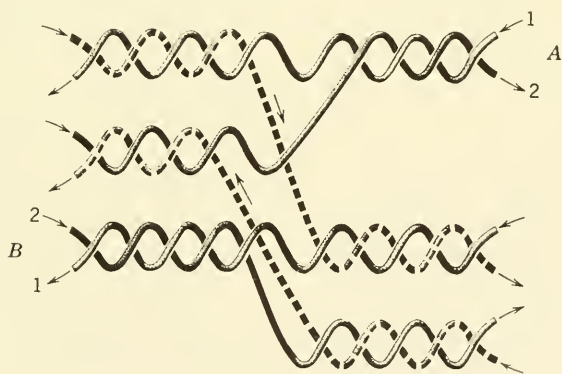


FIGURE 7.12. Proposed model of copy-choice replication of DNA. Assume that A and B represent the DNA double helices of homologous chromosomes, and that 1 and 2 are the complementary strands of each. The newly replicating strands may switch from A to B or from B to A, and as a result each of the new strands will be recombinant.

cal picture of breakage and rejoining of chromatids at the four-strand stage; and (2) that copy-choice is the common basis of all genetic recombination (with sister-strand exchange a necessary cofeature). The great discrepancy between these two points of view underscores how much is yet to be learned about this process.

BIBLIOGRAPHY

- Case, M. E., and N. H. Giles, 1958. Recombination mechanisms at the *pan-2* locus in *Neurospora crassa*. *C. S. H. Symp. Quant. Biol.*, **23**:119-135.
- Chargaff, E., and J. N. Davidson, 1955. *The Nucleic Acids*, Vol. II. New York, Academic Press. (Articles by Swift and by Vendreley.)
- Gall, J. G., 1958. Chromosomal differentiation. In: McElroy, W. D. and B. Glass, (eds.). *The Chemical Basis of Development*, Baltimore, The Johns Hopkins Press, pp. 103-135.
- Hayes, W., and R. C. Clowes (eds.), 1960. *Microbial Genetics*. Society for General Microbiology, 10th Symposium. Cambridge, The University Press, (Articles by Kellenberger and by Pritchard.) 300 pp.
- Kornberg, A., 1960. Biologic synthesis of deoxyribonucleic acid. *Science*, **131**:1503-1508.
- Meselson, M., and F. Stahl, 1958. The replication of DNA in *Escherichia coli*. *Proc. Natl. Acad. Sci. Wash.*, **44**:671-682.
- Mitchell, M. B., 1955. Aberrant recombination of pyridoxine mutants of *Neurospora*. *Proc. Natl. Acad. Sci. Wash.*, **41**:215-220.
- Pontecorvo, G., 1958. *Trends in Genetic Analysis*. New York, Columbia University Press, 145 pp.
- Ris, H., 1957. Chromosome structure. In: McElroy, W. D., and B. Glass (eds.). *The Chemical Basis of Heredity*. Baltimore, The Johns Hopkins Press, pp. 23-69.
- Taylor, J. H., 1957. The time and mode of duplication of chromosomes. *Amer. Nat.*, **91**:209-221.
- Taylor, J. H., P. S. Woods, and W. L. Hughes, 1957. The organization and duplication of chromosomes as revealed by autoradiographic studies using tritium-labeled thymidine. *Proc. Natl. Acad. Sci. Wash.*, **43**:122-128.
- Taylor, J. H., 1959. The organization and duplication of genetic material. *X Intl. Congr. Genet.*, **1**:63-78.
- Taylor, J. H., 1960. Asynchronous duplication of chromosomes in cultured cells of Chinese hamster. *J. Biophys. Biochem. Cytol.*, **7**:455-464.

8 Mutation as a Chemical Process

We left the subject of mutation in Chapter 2 with the concept of the mutable unit as a part of a molecule of nucleic acid which seemed autonomously capable of oscillating with a long phase among a series of chemically different states. This is not an exact picture, because the gene is actually a much more dynamic entity, frequently engaged in its own duplication and exercising its influence on whatever step it controls in the biochemistry of the cell. As a consequence there are several questions we may ask. Does the gene mutate without replicating or is mutation a copy-error occurring rarely during that process? Is mutation a monomolecular event or one subject to the action of the cell's biochemistry with which it is intimately involved? What can be said of the mechanism of mutation?

MUTATION AND GENE REPLICATION

We will first consider whether gene replication is a necessary condition for mutation. Interesting studies have been performed by Novick and Szilard with a device called the chemostat. This consists of a chamber containing a suspension of bacteria (Figure 8.1). Nutrient solution drips in at a slow, constant rate, and at the same rate, the suspension leaks out through an overflow. If the solution entered the chamber faster than the bacteria could divide to maintain their concentration, it would wash them out. But when the bacteria can grow more rapidly than the turnover rate, they exhaust the nutrient that is set as limiting in the incoming solution and thereby maintain their concentration. For every increment of bacteria newly formed, an equal lot is lost in the

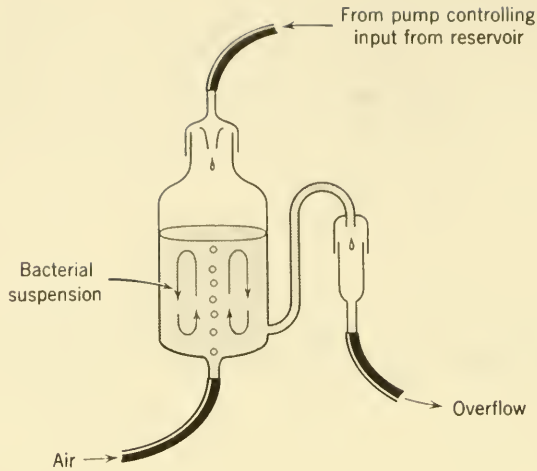


FIGURE 8.1. A chemostat in operation.

overflow. In this steady state the total number of bacteria in the chamber remains constant in accordance with the concentration of limiting nutrient in the input; the rate of growth is also constant and equal to the rate of turnover of the medium which is a function of the rate of input. In a closed system such as a test tube, the concentration of nutrients falls during growth while the concentration of waste products rises. In the open system of the chemostat, since the bacteria immediately use up the incoming nutrient and since waste products are continually lost in the overflow, a steady state of the chemical environment is maintained. In this way, then, the conditions of growth may be accurately controlled and varied at will.

When a mutant arises in the chemostat, it may multiply at the same rate as the parents. If so, its proportion in the population will not increase through growth alone because mutants as well as parents are lost as fast as they are formed. But the proportion of mutants will increase as new mutations occur, and in a linear fashion as long as the number of mutants, M , is so small that it is unnecessary to take back mutation into account. If the growth rate of the mutants is equal to that of the parents, whose number is N , then so long as N is very large relative to M , the rate of increase of mutants will be:

$$\frac{dM}{dt} = aN \quad (8.1)$$

where a is the chance of mutation per parent bacterium per generation,

TABLE 8.1

The Relation of Bacterial Mutation to Division Rate(From Novick and Szilard, 1950, *Proc. Natl. Acad. Sci.*, **36**:708)

Generation Time, Hours	Mutation Rate per Bacterium per Generation	Mutation Rate per Bacterium per Hour
2	2.5×10^{-8}	1.25×10^{-8}
6	7.5×10^{-8}	1.25×10^{-8}
12	15.0×10^{-8}	1.25×10^{-8}

and t is time. At any time, then, the proportion of mutants is:

$$\frac{M}{N} = at + C \quad (8.2)$$

where C is the initial proportion of mutants at time zero (t_0). This is the equation for the curve in Figure 8.2.

Novick and Szilard changed the rate of growth of bacteria in the chemostat by changing the flow rate, and observed that the mutation rate, a , was an inverse function of the rate of growth (Table 8.1). The relation held for a broad range of growth rates. It seems, then, the mutations occur at a constant rate in time, irrespective of the number of generations that take place in any interval. At first glance this might seem to suggest that mutation is not a function of gene replication; it might even occur while the gene is not replicating. But it is likely that, when bacteria grow at different rates, the period during which the gene is replicating may be prolonged or shortened accordingly. Perhaps mutations have a measurable probability of taking place only while gene replication is going on. If this were so, then mutation could be constant with time and yet could occur as a copy-error.

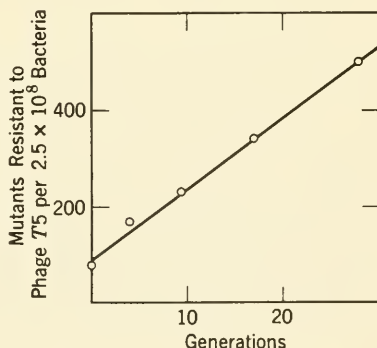


FIGURE 8.2. The rate of increase of mutants in the chemostat (from Novick and Szilard, 1950, *Proc. Natl. Acad. Sci. Wash.*, **36**:708).

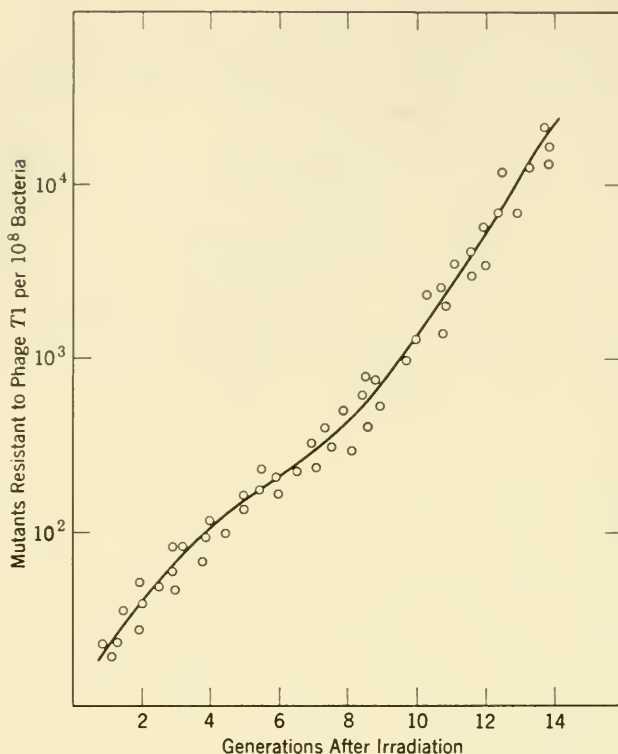


FIGURE 8.3. The relation of bacterial mutation induced by ultraviolet light to the rate of division at various temperatures; the same curve describes the results at 37, 25, and 16°C (from Witkin, 1953, *Proc. Natl. Acad. Sci. Wash.*, **39**:427).

This concept agrees with other work indicating that mutation is fastest when cell divisions are taking place, and that the temperature coefficients of the two processes are the same (Figure 8.3). Yet Stubbe, in Germany, long ago showed that mutations could occur even in stored seeds. The rate was, however, not constant but increased with time, suggesting that as aging progresses the mutation rate changes in accordance with changes in metabolism. Perhaps the genes slowly replicated in the stored seeds at an increasing rate. It has also been shown that non-dividing bacteria can mutate at a slow rate which *does* remain constant with time. The mutation rate is a function of the ease with which various sugars can be utilized, and metabolism may be slowed down to such an extent by low temperature or starvation that new mutations cannot be observed to take place. By the use of chloramphenicol, which in-

hibits protein synthesis, these mutations could be prevented, suggesting that they occurred as a result of the metabolic turnover of macromolecules. Of course, it is possible that some mutations may still take place in nonreplicating genes by, for example, exchange reactions; we may suspect, however, that most spontaneous mutations are copy-errors in the course of gene replication.

Similar conclusions may be drawn from studies of induced mutation. Viruses, for example, in the free state cannot be shown to mutate spontaneously at a detectable rate, and they mutate only rarely even after treatment with radiation. But if they are allowed to infect a cell, they mutate with a small chance during their multiplication and abundantly if the infected cell is irradiated. Even irradiating the bacterial cell alone before infection induces mutations in the untreated viruses that later multiply in it. Perhaps this is due to recombination between virus and host, but we may suspect that viruses need to duplicate in order to mutate, and furthermore, that mutation may involve the incorporation of unusual precursors during DNA replication. In this connec-

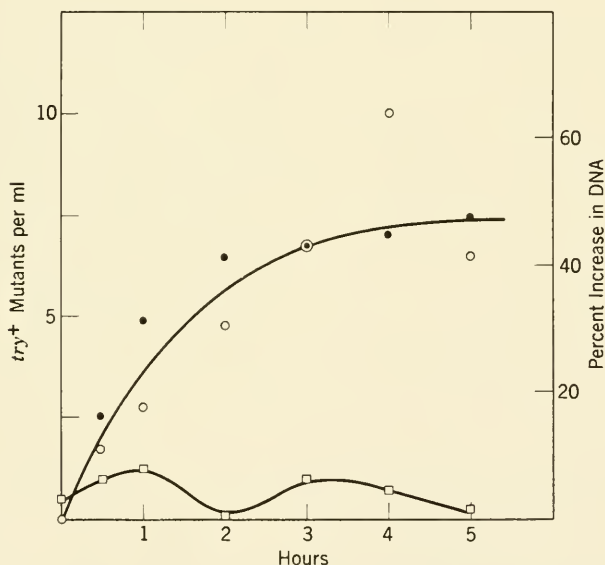


FIGURE 8.4. The dependence of mutation on the residual replication of DNA in *Salmonella typhimurium* (try^-) put in the presence of chloramphenicol but no tryptophane where the viable cell number remains constant. The mutagen, 2-aminopurine, was added at the beginning of the experiment and after 2 hours when the DNA had almost ceased replication (after Nakada, Strelzoff, Rudner, and Ryan, 1960, *Zt. Vererb.*, **91**:210-213).

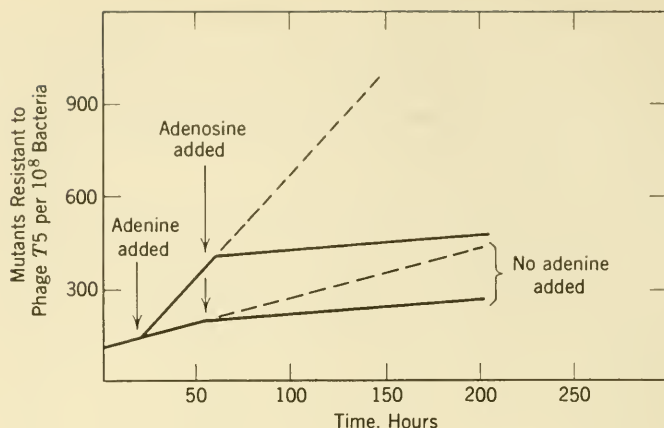


FIGURE 8.5. Simulacre of the effect of adenosine on adenine-induced and spontaneous mutation of bacteria in the chemostat (after Novick and Szilard, 1952, *Nature*, 170:927).

tion, it has been shown in bacteria that growth and division, with the inevitable DNA synthesis that accompanies them, are prerequisite for the mutagenic action of purine analogues. One of these, 2-amino purine, induces mutations while DNA is replicating but not after it has nearly ceased (Figure 8.4). Furthermore, when the purine, caffeine, is used as a mutagen, its effect can be shown to be decreased by chloramphenicol during the first few hours of treatment in proportion to the decrease in the rate of DNA synthesis that occurs. It seems, then, that unusual purines and purine analogues, if they are not incorporated themselves, somehow affect the incorporation of bases into newly synthesized DNA; as a consequence, replication is necessary before the mutations can occur.

INCORPORATION OF UNUSUAL NUCLEOTIDES AND COPY ERRORS

Some studies on mutation made in the chemostat have shown that purines, such as caffeine and adenine, are mutagenic when they are continually supplied to the medium, while some ribosides, such as adenosine and guanosine, can reverse this effect. Adenosine even reduces spontaneous mutation in the absence of adenine, but it cannot eliminate it entirely (Figure 8.5). Therefore, we know that there are likely to be at least two mechanisms for spontaneous mutation. The one that is

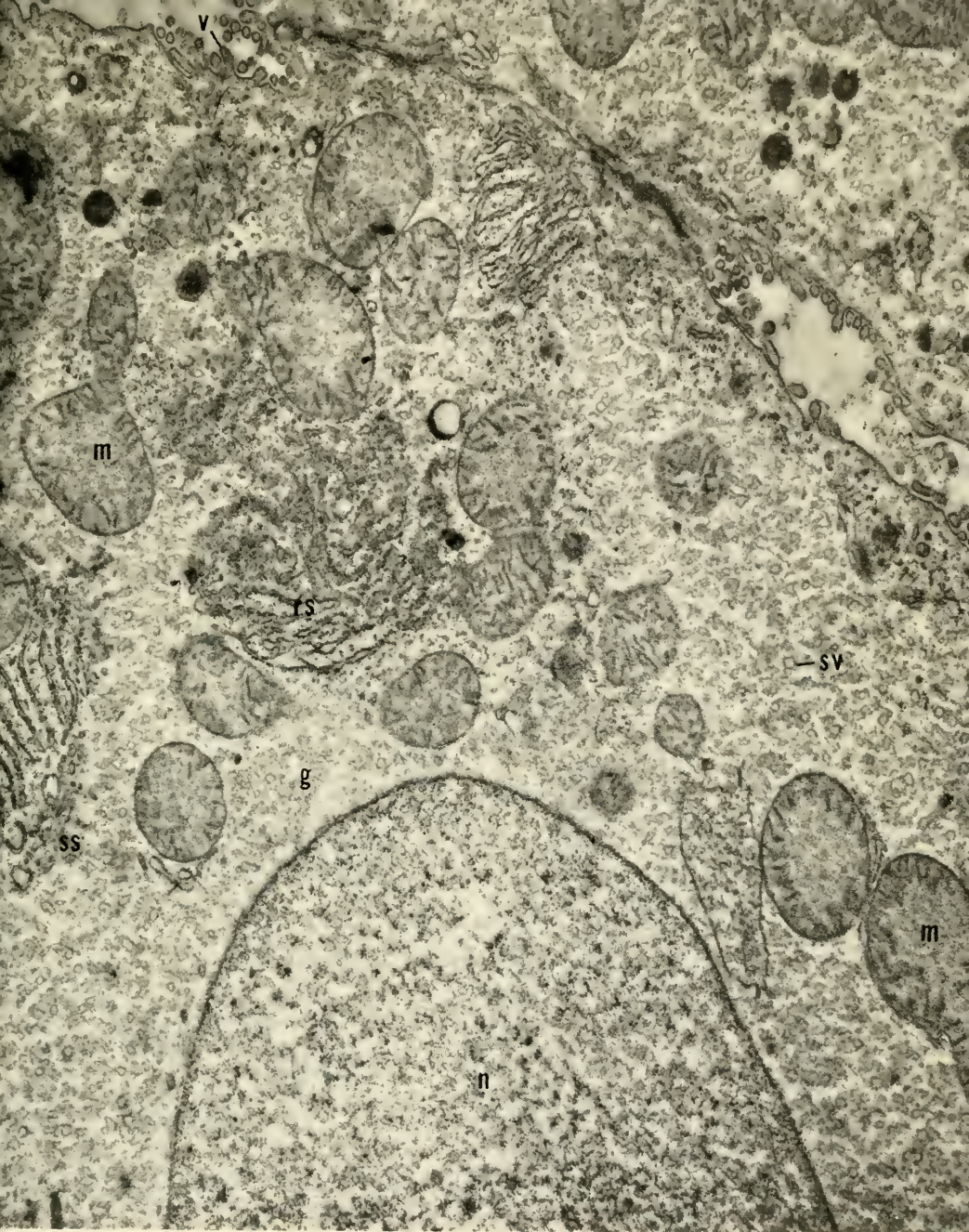


PLATE I. Port of a liver cell (rot) showing the nucleus (n), mitochondria (m), rough surfaced membranes (rs) of the endoplasmic reticulum with ribosomes, smooth surfaced membranes (ss), and small vesicles (sv). There is a heavy glycogen deposit (g) in the cytoplasmic matrix. The free surface of the cell shows numerous microvilli (v). 14,000 X. (Courtesy of G. E. Palode.)



PLATE II. Sectioned cell of *E. coli*. Note bacterial wall (w) and plasma membrane (p). The cell is packed with ribosomes except in the nuclear region where DNA-containing fibers are seen. No nuclear membranes or membranous cytoplasmic organelles are apparent. 53,000 X. (Courtesy of E. Kellenberger.)

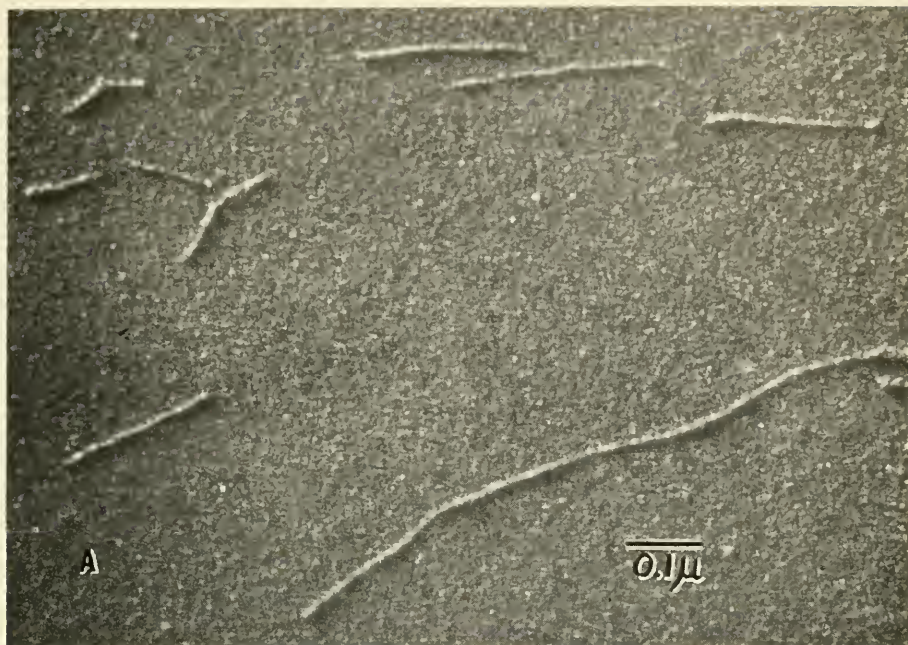


PLATE III. (A) Fibers of DNA (deoxyribonucleic acid) from salmon sperm. 100,000 $\times$. (Courtesy of Cecil E. Hall.) (B) Fibers of DNP (deoxyribonucleoprotein) from calf thymus. 100,000 $\times$. (Courtesy of Cecil E. Hall.)

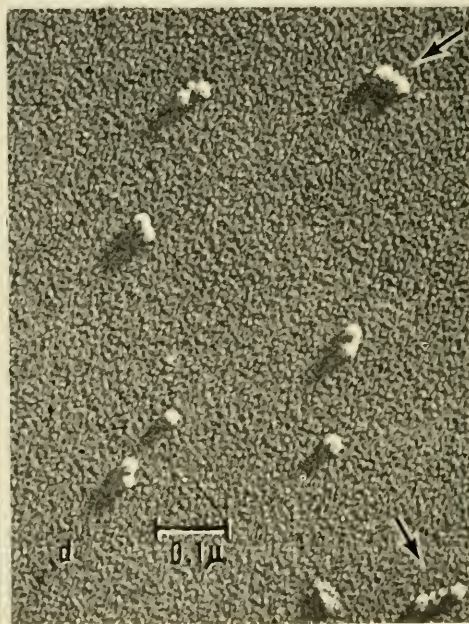
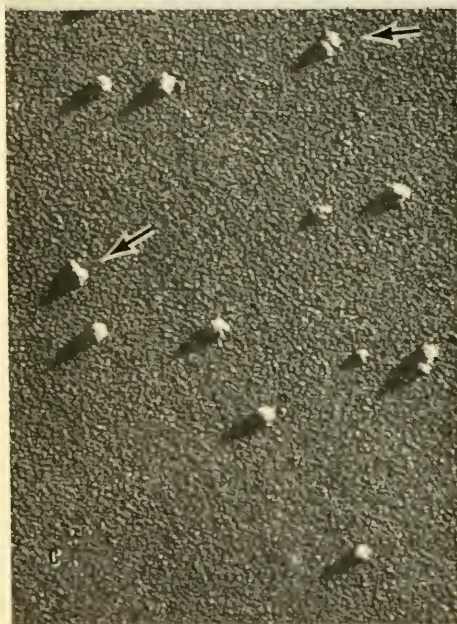
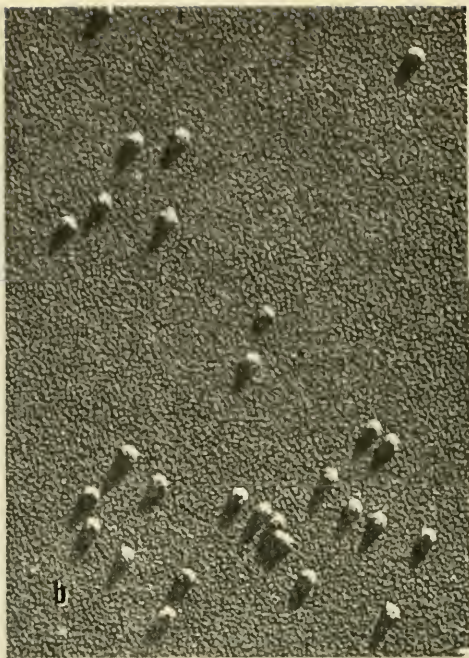
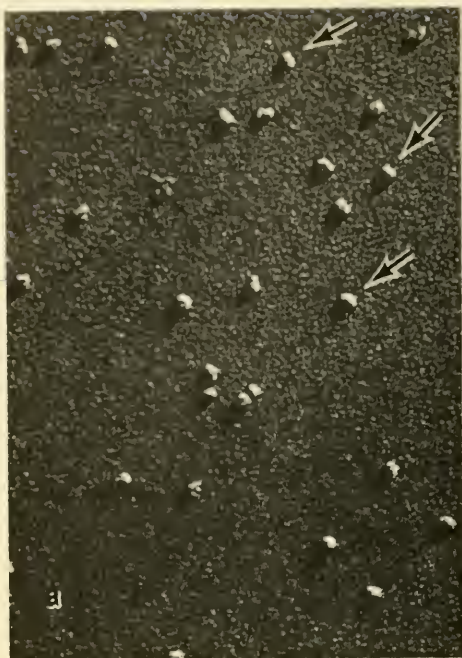


PLATE IV. Ribonucleoprotein particles (RNP) from *E. coli*. 100,000 $\times$. (a) RNP particles from a 30-s preparation. The arrow indicates a typical asymmetrical particle. (b) RNP particles from a 50-s preparation. (c) RNP particles from a 70-s preparation. These specimens also show 30 and 50 s particles. The 70 s particles, such as are indicated by arrows, show a substructure indicating doublet-ness. (d) RNP particles from a 100-s preparation. The 100 s particles are dimers of the 70 s. (From Hall and Sloyer, 1959, *J. Mol. Biol.*, 1:329.)

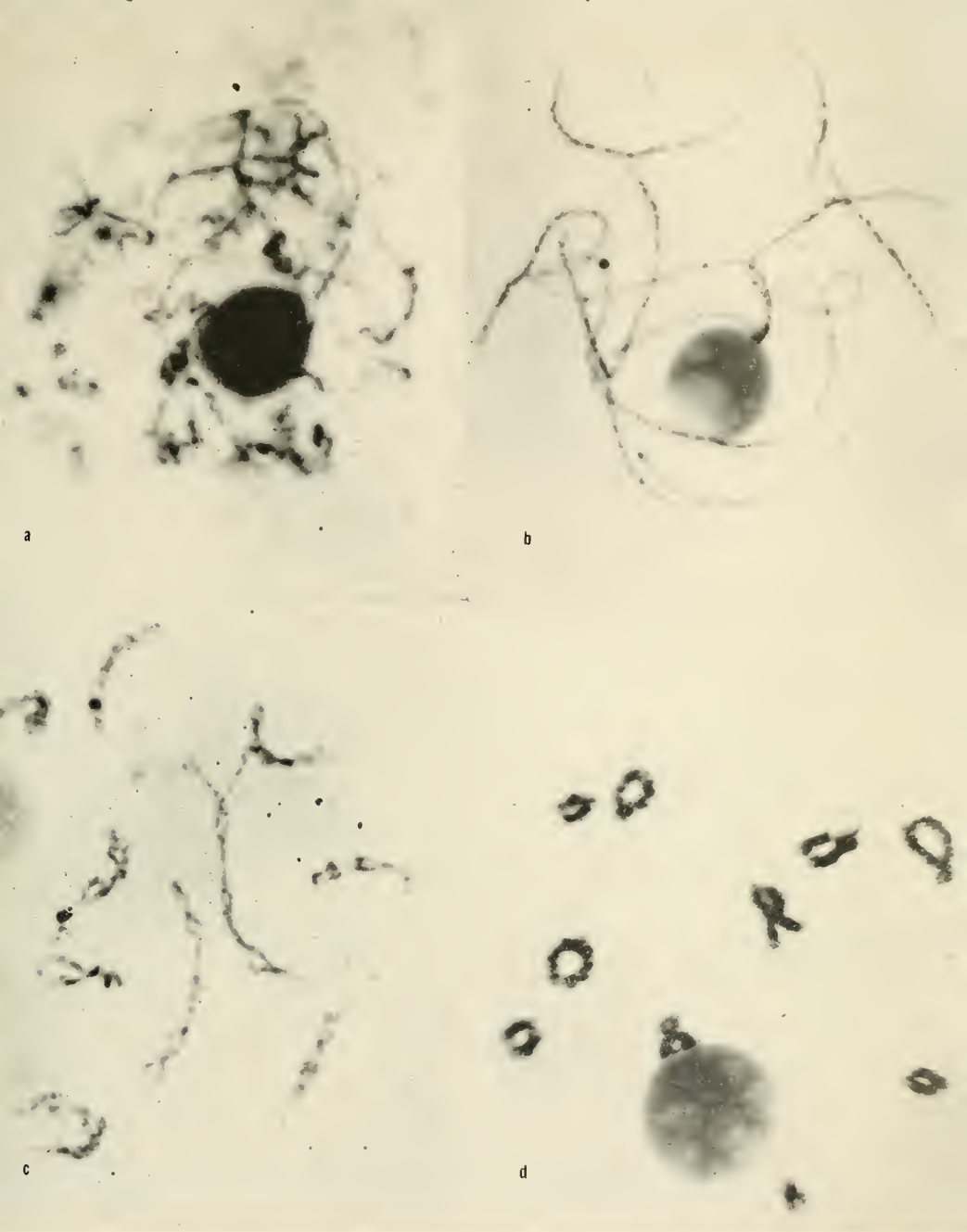


PLATE V. Meiotic chromosomes of *Zea mays*, the corn plant, stained with acetocarmine. (a) Leptonema. (b) Pachynema. (c) Diplonema. (d) Diakinesis. (Courtesy of M. M. Rhoades. Figure b is by D. M. Morgan.)

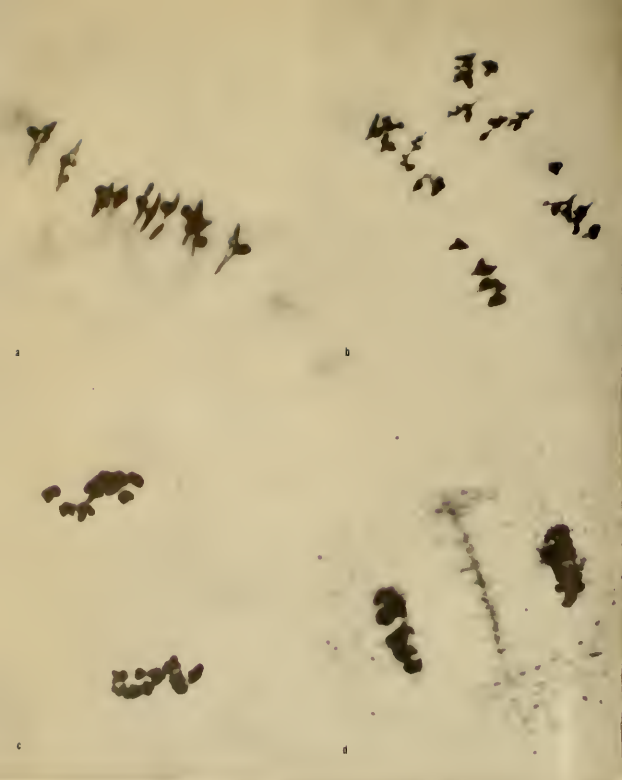


PLATE VI. Meiotic chromosomes of *Zea mays*, the corn plant, stained with acetocarmine. (a) Metaphase I. (b) Anaphase I. (c) Telophase I. (d) Meiotic interphase. (Courtesy of M. M. Rhoades.)



PLATE VII. Meiotic chromosomes of *Zea mays*, the corn plant, stained with acetocarmine. (a) Early prophase II. (b) Late prophase II. (c) Metaphase II. (d) Anaphase II. (Courtesy of M. M. Rhoades.)



PLATE VIII. Salivary gland chromosomes of *Drosophila melanogaster*, showing the bonding pattern. The arms are marked with their conventional nomenclature. (Courtesy of B. P. Kaufmann.)

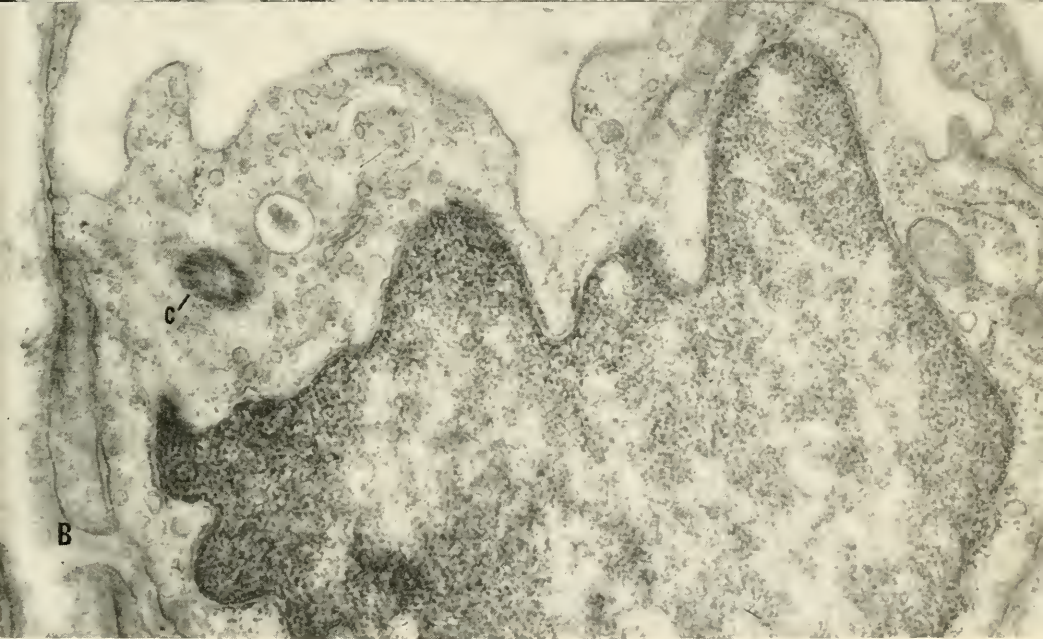
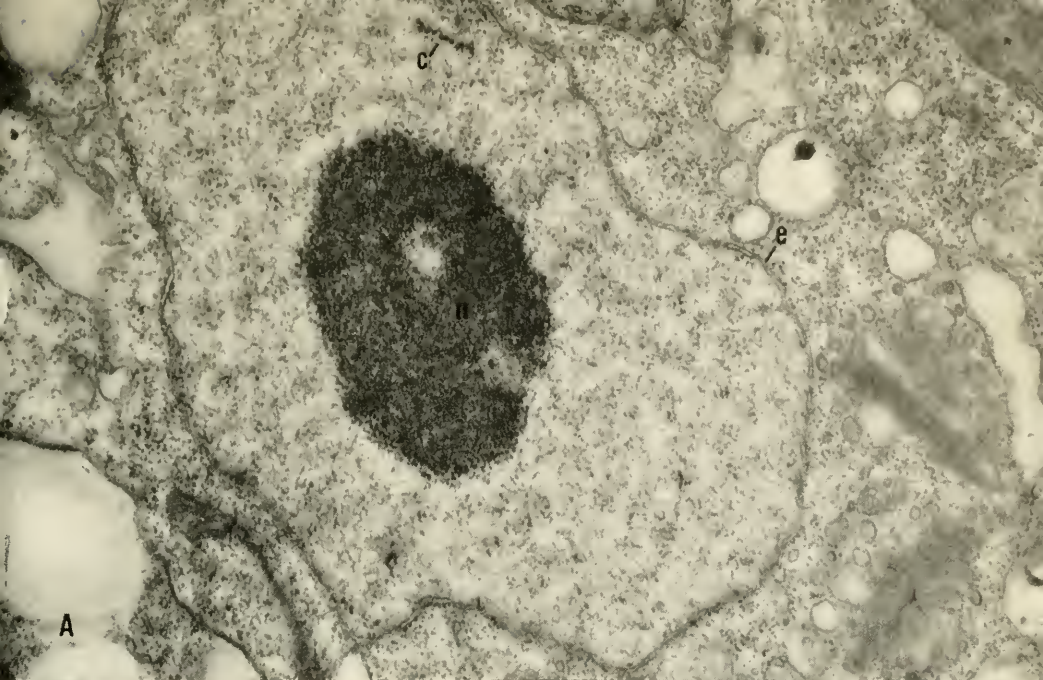


PLATE IX. (A) Nucleus of *Chlamydomonas* showing the nuclear envelope (e) with its two membranes and the dense granular nucleolus (n). The condensed structure at c probably represents a part of a chromosome in longitudinal section. 40,000 X. (Courtesy of R. Sager and G. E. Palade.) (B) Nucleus of an endothelial cell (rat) with chromosomes condensed and located mainly near the nuclear envelope. Note at c a centriole sectioned almost transversely. 34,000 X. (Courtesy of G. E. Palade.)

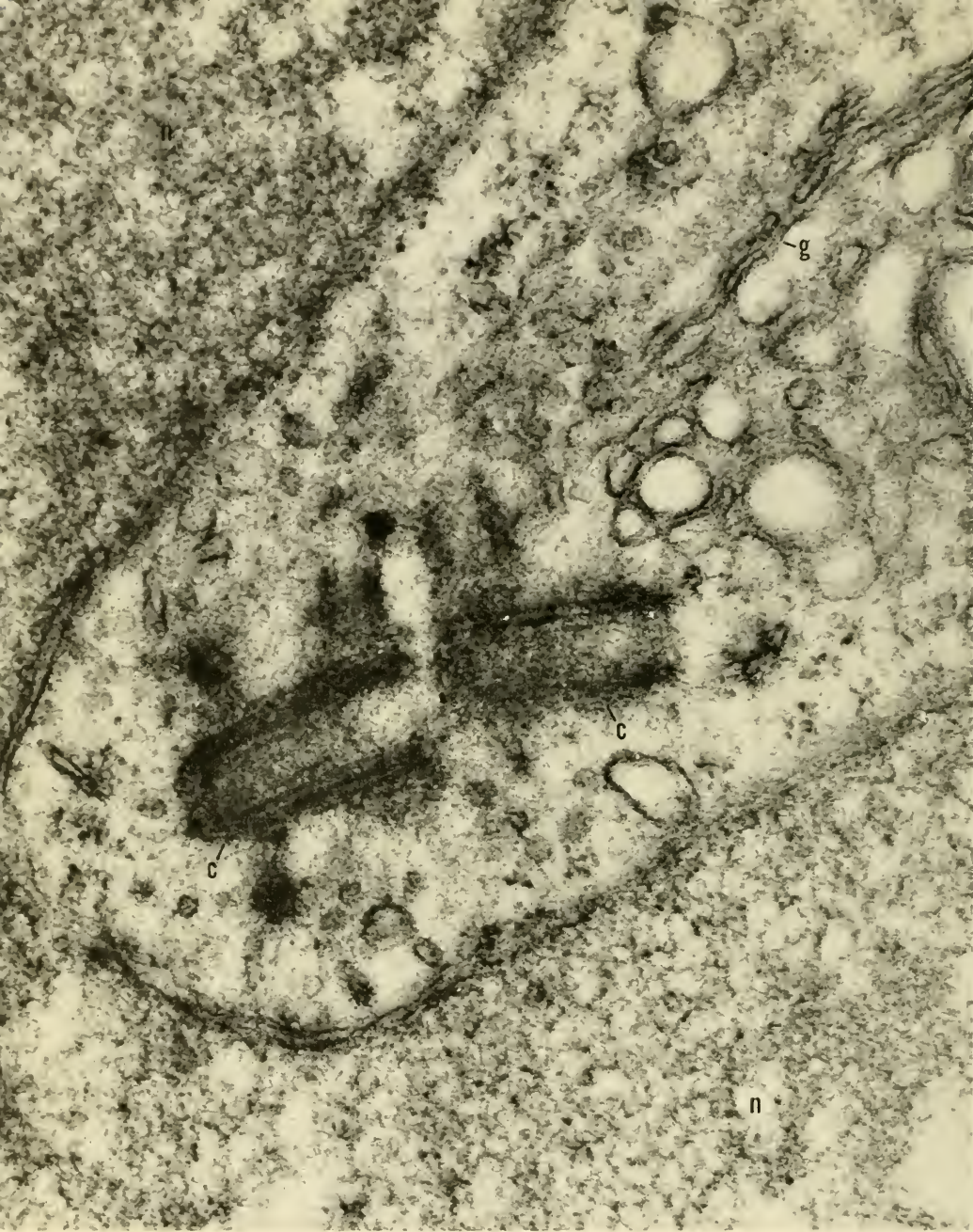


PLATE X. Centrioles (c) lying in the cytoplasm in a deep indentation of the nucleus (n) close to the Golgi complex (g). Both centrioles are sectioned longitudinally. 86,000 X. (Courtesy of E. de Harven.)

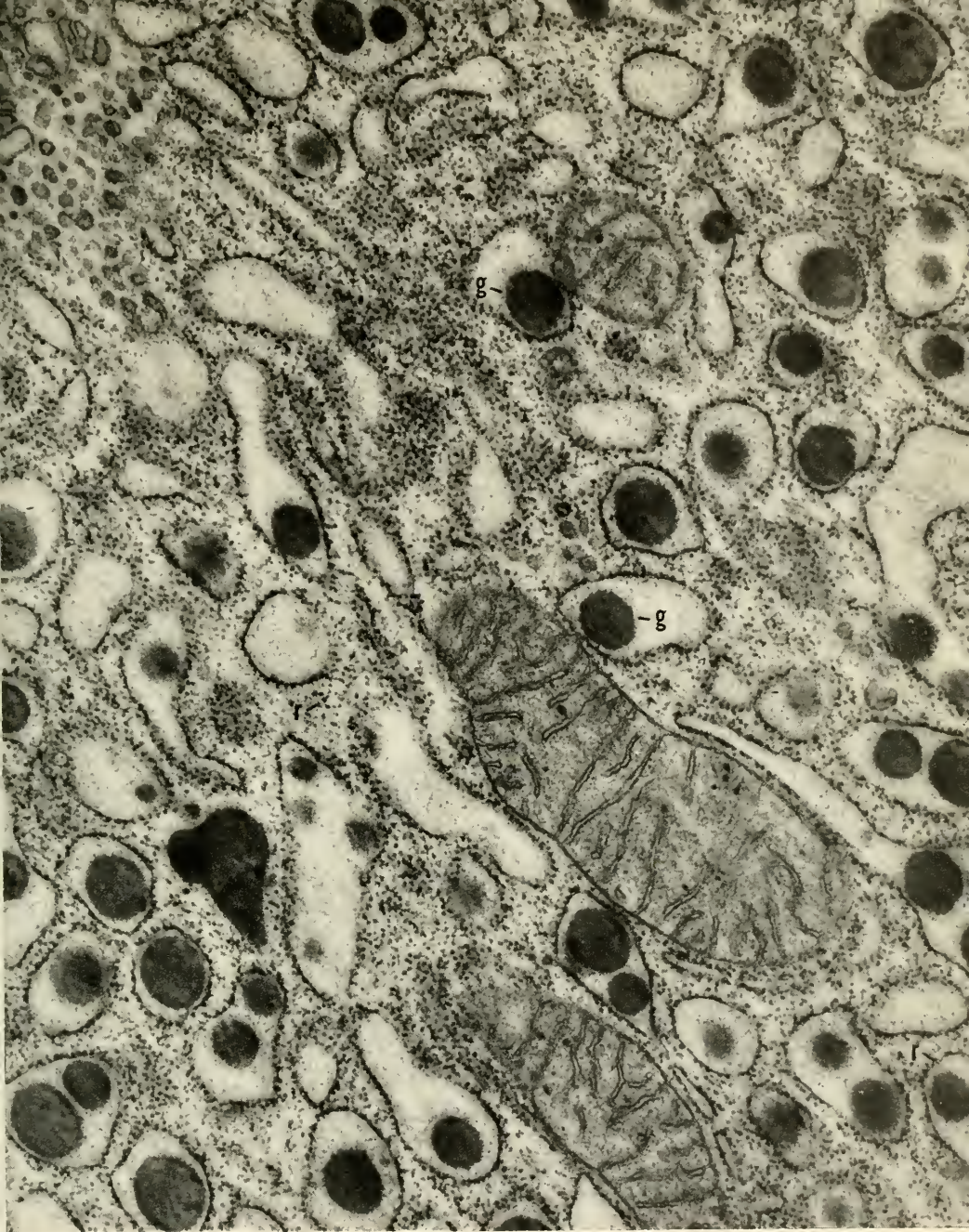


PLATE XI. Endoplasmic reticulum of a pancreatic acinar cell (guinea pig) showing newly formed proteins collecting in granules (g) in the network of tubules leading eventually to the cell membrane. The dense ribosomal particles (r), sites of protein synthesis, are found in the cytoplasmic matrix and on the membranes of the endoplasmic reticulum. 40,000 X. (Courtesy of G. E. Palade.)

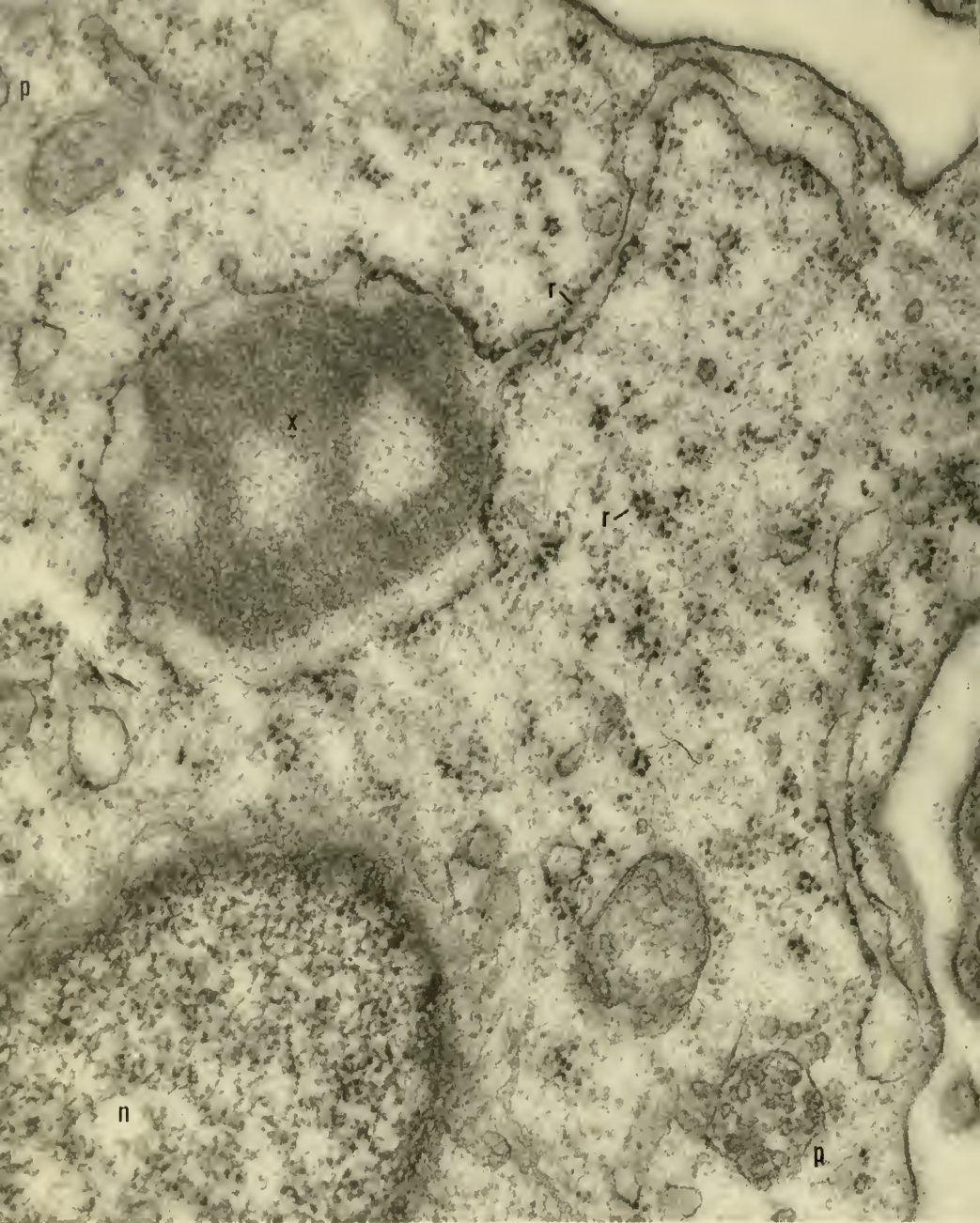


PLATE XII. Endoplasmic reticulum in a kidney epithelial cell (rat glomerulus) showing a cisterna distended at one end to form a large vacuole probably containing newly synthesized protein (x). Ribosomes (r) are seen on the membranes and in the cytoplasmic matrix. The nucleus (n) and two "polko dot bodies" (p) (multivesicular bodies), concerned with transport into the cell, are also present. 60,000 $\times$. (Courtesy of M. G. Farquhar and G. E. Palade.)

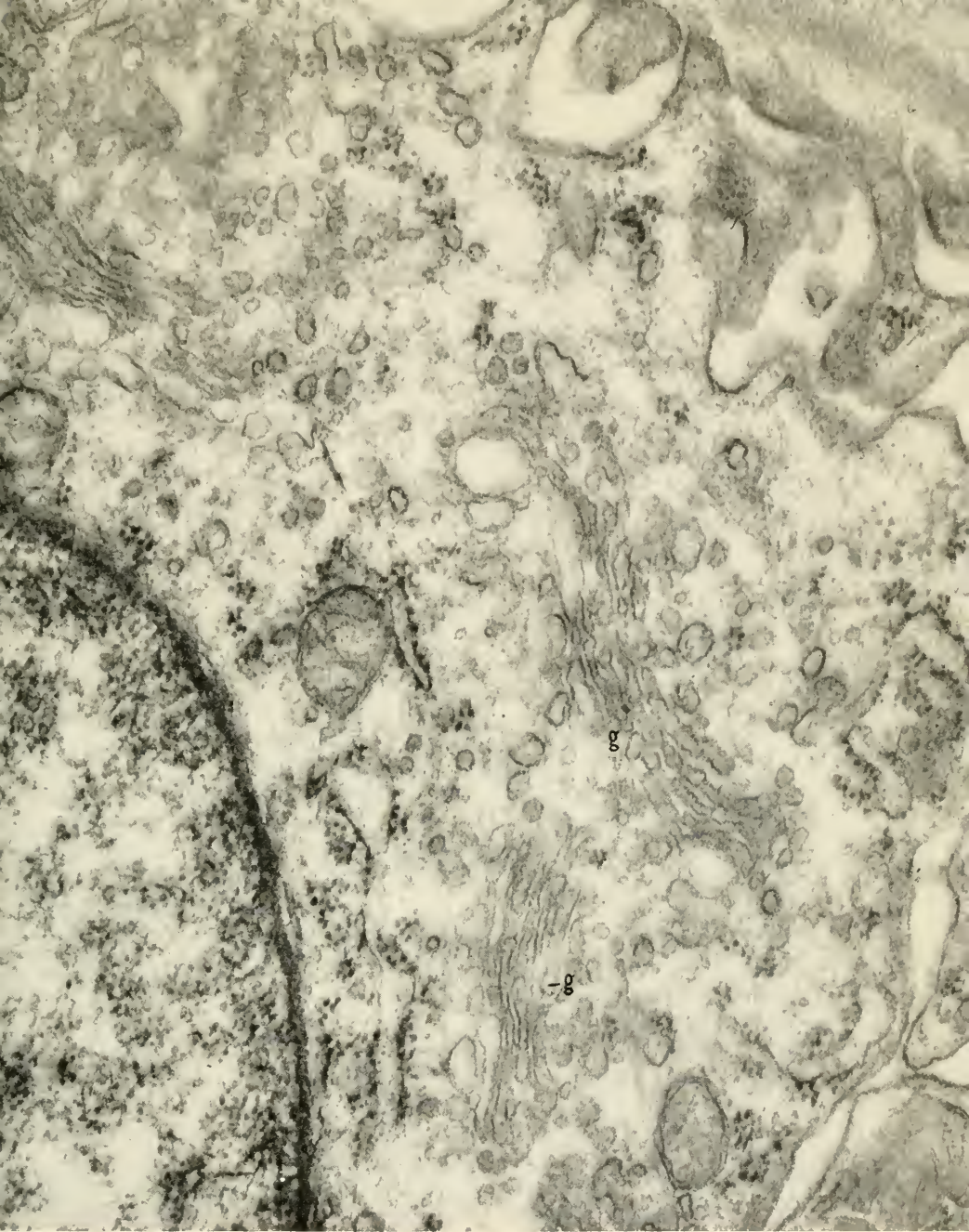


PLATE XIII. A kidney epithelial cell (not glomerulus) showing several stacks of Golgi membranes (g), each consisting of a pile of flat vesicles with small round vacuoles at each end. Note similarity to the Golgi complex of *Chlamydomonas* in Plate XVI. 62,000 X. (Courtesy of M. G. Farquhar and G. E. Palade.)

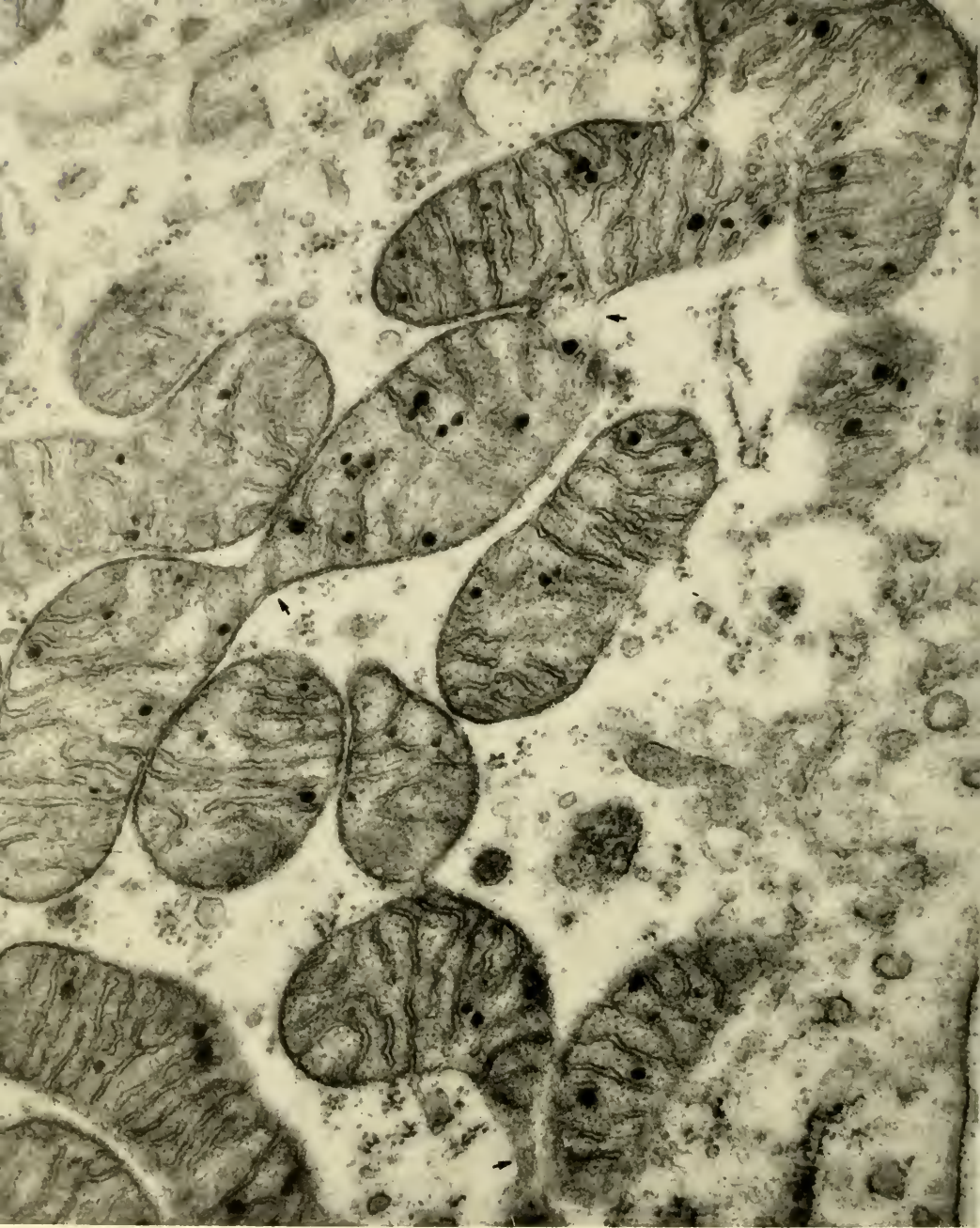


PLATE XIV. Mitochondria of a pancreatic cell (guinea pig) showing the continuous outer membrane, the inner membrane to which the cristae are attached, and the matrix which is much denser than the surrounding cytoplasm. Mitochondria which appear to be dividing may be caught in a twisted configuration (arrows). 53,000 X. (Courtesy of G. E. Palade.)



PLATE XV. Part of the chloroplast of *Chlamydomonas* showing the chloroplast envelope (e), the discs (d marks the edge of one of them) partially stacked into grana (g), and a starch grain (s) all embedded in a granular chloroplast matrix. The cell membrane (m) and the nuclear envelope with one of its pairs (p) can also be seen. 100,000 X. (Courtesy of R. Sager and G. E. Palade.)

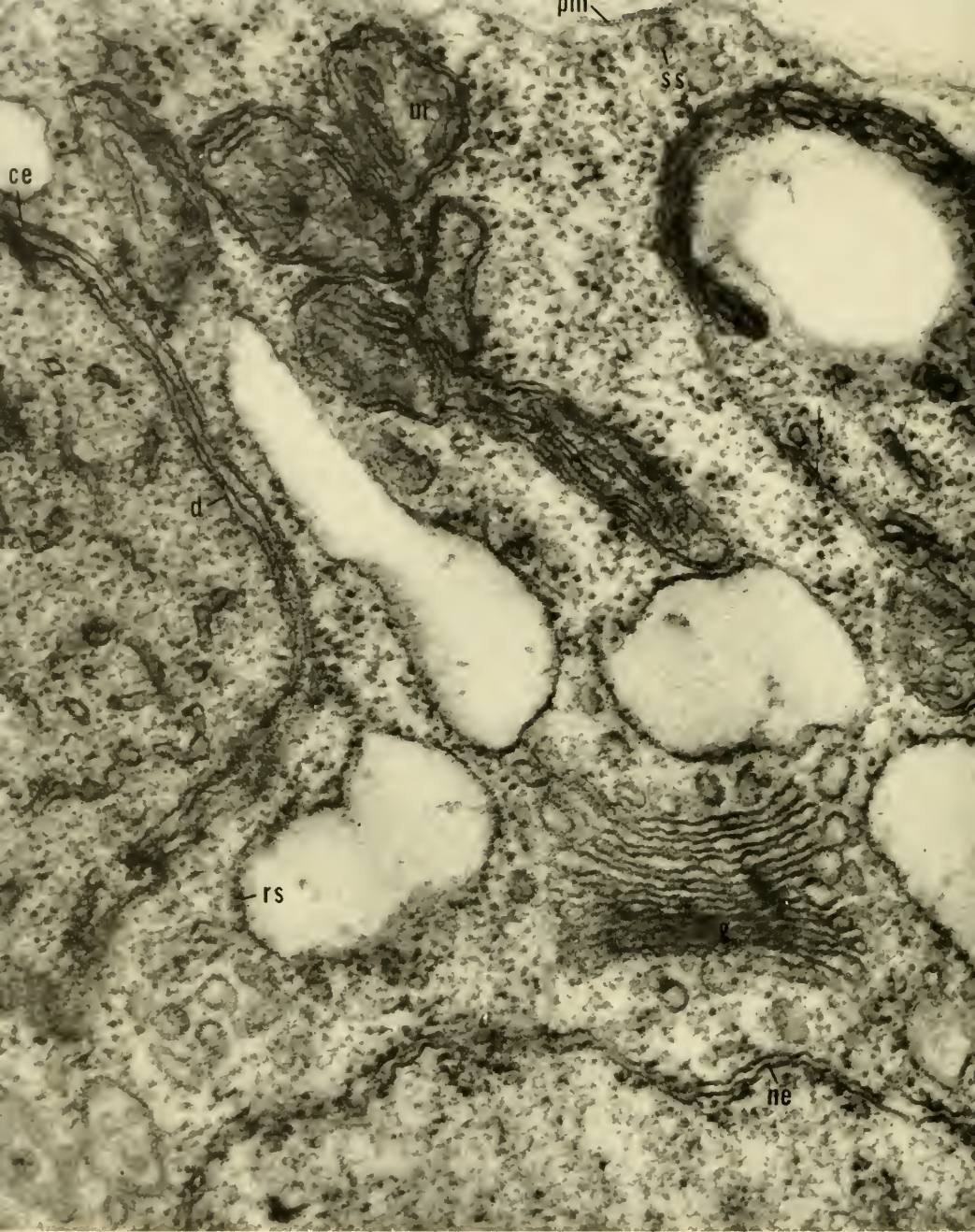


PLATE XVI. Port of a cell of *Chlamydomonas* showing the extensive use of membranes for the cell boundary (pm), the nuclear envelope (ne), the chloroplast envelope (ce), the chloroplast lamellae or discs (d), mitochondrial outer membranes and cristae (ot m), the Golgi complex (g), smooth-surfaced (ss) and rough-surfaced (rs) elements of the endoplasmic reticulum, and for other organelles as yet unidentified. The paucity of chloroplast lamellae corresponds to the small amount of chlorophyll present in this mutant cell. 80,000 X. (Courtesy of R. Soger and G. E. Polode.)

reversible would seem to involve substances like adenine and adenosine which are naturally occurring metabolites. In some competitive way, too much adenine may prevent the normal metabolism of adenosine so that mutation becomes likely. The achievement of a proper balance in the concentration of substances like these may be one of the regulatory mechanisms that determine the mutation rate. It is strange that pyrimidines have been found not to be mutagenic and, furthermore, that *deoxyribosides* are not antimutagens.

Nonetheless, some unnatural analogues of pyrimidines, when supplied to the cell, are actually incorporated into DNA. For example, 5-bromouracil quantitatively replaces thymine in DNA and is mutagenic. But it must be emphasized that even when almost 100 per cent of the thymine has been replaced by 5-bromouracil, most of the genes do not mutate and appreciable numbers of cells or phage survive. This implies that the incorporation itself does not constitute the mutation, for a gene which has incorporated 5-bromouracil cannot duplicate gene replicas containing that abnormal base when it is no longer supplied in the medium. Therefore, the replica of the gene containing that analogue (Figure 8.6) must usually incorporate adenine at the corresponding site; at the next replication, thymine would be incorporated in the new strand opposite the adenine to restore the original condition. But it is likely

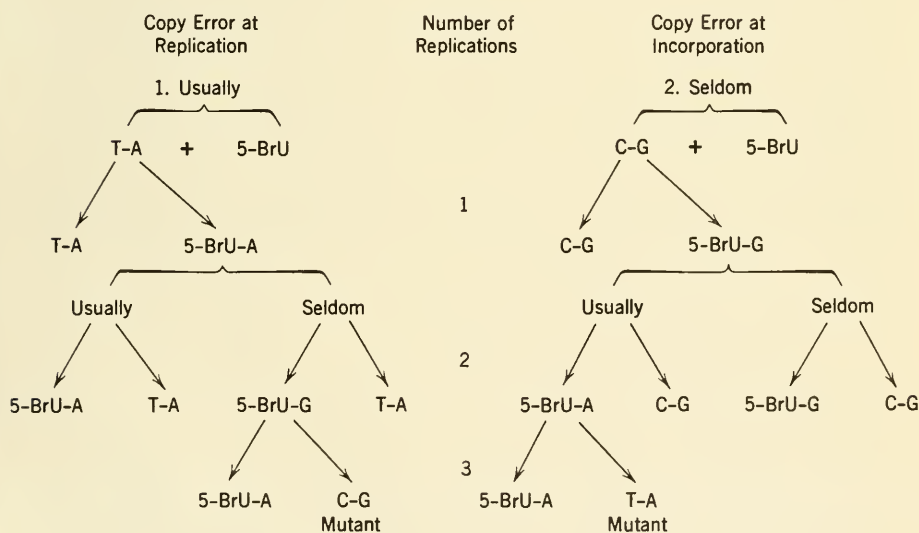


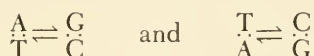
FIGURE 8.6. A speculation on the effect of substituting 5-bromouracil for thymine in replicating DNA.

that once in a while some other base is incorporated opposite 5-bromouracil during replication. The mutation would then be completed when that base, let us say guanine, chose its normal partner, cytosine, at the next replication. It seems clear that DNA carrying the base analogue cannot function to give the mutant phenotype before the mutation is genetically completed. Recent studies with synchronously replicating bacteria show that two replications after incorporation are required before 5-bromouracil-induced mutants can be detected.

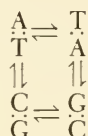
As long as DNA containing 5-bromouracil continues to replicate, it will continue to mutate; such mutability in a growing population would, however, soon disappear unless the proportion of genes with 5-bromouracil were maintained at a high level by continued provision of the analogue. Since this hypothesis of incorporation and copy-error assumes that 5-bromouracil sometimes chooses as a partner guanine rather than adenine, it must sometimes replace cytosine at incorporation, with the consequences shown in case 2 of Figure 8.6. We must further consider that base analogues may be incorporated by exchange reactions in the absence of replication.

In any event, we may conceive of some mutations as consisting of at least two steps. The first involves the incorporation of an unusual base in a certain position on one strand of the double helix of DNA, and the second consists of replication on this template. The base may be unusual in either of two ways. First, it may not normally be synthesized by the cell but may require the action of some agent such as ultraviolet light for its production. In this event, the unusual base can be incorporated only during the short time it is present in the cell. This initial incorporation would not constitute the complete mutation; it would be followed by a replication which involves the introduction of a novel but normally synthesized base at the affected site in the new strand, just as was the case with 5-bromouracil shown in Figure 8.6. Alternatively, the incorporated base may be unusual only because, although it is normally synthesized by the cell, it occupies a novel position in that, in the parental DNA, another base was present at that site. Errors in incorporation of this sort might occur with increased chance in the presence of agents which break the hydrogen bonds of DNA and separate the strands. Temperature does this, and heat shock is known to induce mutation. Furthermore, certain chemicals of the right dimensions and groupings might insert themselves between the two chains and separate them. A large imbalance in the proportions of nucleotide precursors might also engender this kind of error. In any event, replication would be needed, first for the incorporation of the unusual base, and again for the stabilization of the new pattern by the inclusion of a complementary base in the next generation of polynucleotide strands.

There are two kinds of base replacements that can be made by pairing mistakes. In the first case a purine may be replaced by another purine, or a pyrimidine by another pyrimidine (transitions). In the second case a purine may be replaced by a pyrimidine, and vice versa (transversions). The consequences are quite different. In the first case the only possible transitions of base pairs are:



(Remember that the pairs $\begin{array}{c} \text{A} \\ \vdots \\ \text{T} \end{array}$ and $\begin{array}{c} \text{T} \\ \vdots \\ \text{A} \end{array}$ and $\begin{array}{c} \text{G} \\ \vdots \\ \text{C} \end{array}$ and $\begin{array}{c} \text{C} \\ \vdots \\ \text{G} \end{array}$ would, in these inverted positions, give a different code to the DNA because of their relation to neighboring base pairs.) In the second case, the following transversions are possible:



They can be achieved through the incorporation by mistake of a purine in place of a pyrimidine, or vice versa. In the case illustrated in Figure 8.7, guanine is incorporated opposite adenine instead of thymine. The second replication results in a mutant DNA where, in the site once occupied by the base pair A:T, we now have C:G.

All of the transpositions of both types are illustrated by double arrows because the mutations should be reversible. However, if some mutagen acted to cause a replacement of a pyrimidine by a pyrimidine or a purine by a purine, then it could induce the first but not the second kind of base change, and it could not reverse a mutation of the second type. For example, the change T:A to C:G, shown in the first illustration of Figure 8.6, could be both induced and reversed by such a mutagen, but the

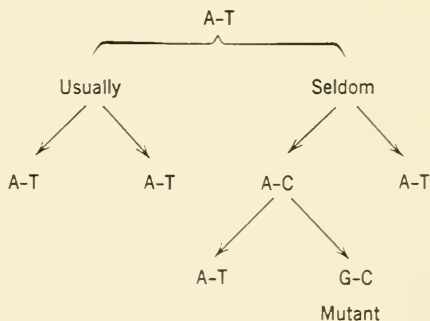


FIGURE 8.7. Example of a mutation resulting from the incorporation by mistake of purine in place of a pyrimidine during the replication of DNA.

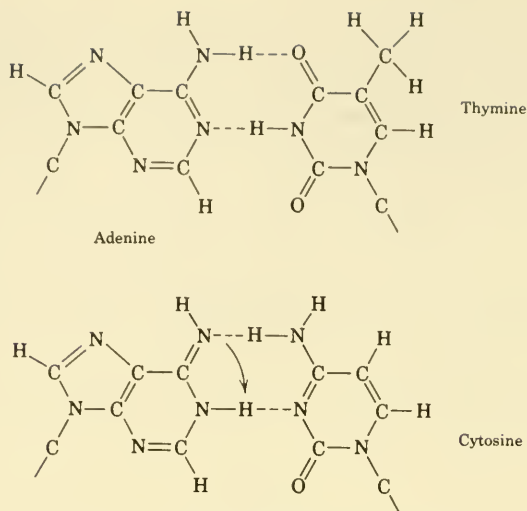


FIGURE 8.8. The pairing possibilities of adenine in its two tautomeric states.

change A:T to C:G in Figure 8.7 could not. In the latter case, the mutagen could convert C:G only into T:A. Yet all these changes should be spontaneously reversible.

Some evidence relating to these speculations has been provided by Freese. Both 5-bromouracil and 2-amino-purine (which might substitute for adenine in DNA) induce mutations in bacteriophage *T4*. When mutants so produced are treated with either chemical they are induced to back-mutate at a low rate. But these mutagens cannot reverse mutations induced by proflavin and are able to reverse only about 10 per cent of spontaneous mutations. Nevertheless, all of these mutations revert spontaneously. We may presume, therefore, that 5-bromouracil and 2-amino-purine cause mutations of the first kind (transitions), whereas most spontaneous and proflavin-induced mutations are transversions. It is not known whether these two mechanisms bear any relation to the spontaneous bacterial mutations allowed and prevented by adenosine in the chemostat. Nor is it understood how suprastructural factors determine the likelihood of copy-errors at replication. We may be sure that they do, for, as was observed in Figure 6.7, the incorporation of base analogues resulted in a large number of different instabilities peculiar to the sites affected. No doubt the neighboring nucleotides in some way determine the chance of incorporation or the chance that an unusual choice of a base partner will be made at replication, or both. It is even possible that an analogue may choose a partner in error with a lower fre-

quency than does the base it replaced; it would therefore reduce the chance of mutation and make the gene more, rather than less, stable.

Although there is still much to be learned, we no longer need conceive of mutant alleles as existing in different thermodynamic energy troughs for completely unspecified reasons. Watson and Crick, in originally proposing their model, noted that the bases may sometimes be in a rare tautomeric state. Adenine, for example, oscillates between the two states shown in Figure 8.8. In the first, or common, state it is capable of fitting opposite thymine in the double helix, but in the second, or rare, state it is compatible only with cytosine, of the four bases. If it were in this rare state at the moment of duplication, it would be likely to choose cytosine as its mate, and this base would be incorporated in the new polynucleotide strand. At the next replication, guanine would pair with cytosine and a transitional change in base order would have been accomplished. In comparable ways, molecular mechanisms must be envisaged for mutations that occur by transversion.

POINT MUTATIONS VERSUS GROSSER CHANGES IN THE GENE

The mechanisms of point mutation discussed above have all presumed that the molecular event was at the level of nucleotide pairs and involved a base substitution. Since many mutations can be localized well within the extent of the functional gene, this presumption seems a valid one. Nonetheless we must leave open the possibility that mutations may be of many kinds. Some may involve breaks in the backbone of the polynucleotide strands. Such breaks can be induced by ionizing radiations and by radiomimetic alkylating agents, such as the sulphur and nitrogen mustards and the epoxides. Double breaks at the same level, one in each complementary strand, would not heal with a switching between the two strands because of the polarity of structure which runs in an opposite direction in each polynucleotide chain of the DNA double helix. If they did not heal to restore the original continuity, they would cause a fragmentation of the DNA molecule. But breaks in different positions in the two strands and breaks in the same strand would not immediately cause degradation of the DNA because the fragmented strands would remain attached to each other by hydrogen bonds, and the breaks would remain cryptic until these bonds were parted. Urea will separate the twin strands of DNA, and it has been shown to reveal breaks induced by treatment with X-rays or alkylating agents (Figure 8.9). One consequence of double breaks in the same strand might be a deletion, yielding defects which would occupy loci including one or more

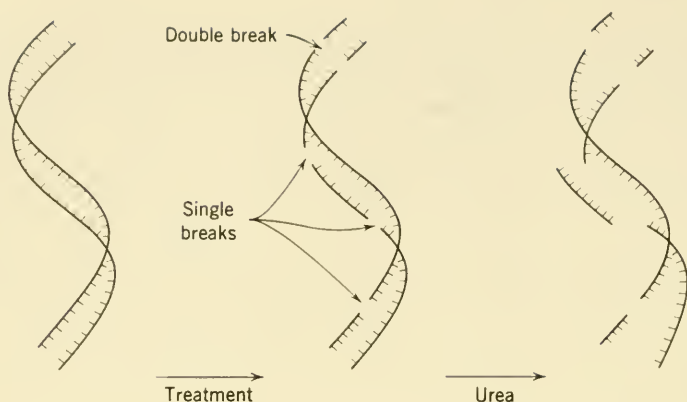


FIGURE 8.9. A diagram of the breakage of the sugar-phosphate backbone of DNA by alkylating agents or X-rays.

nucleotide pairs and producing stable mutants which would not revert. Evidence exists for such occurrences within the structure of the gene. Other conceivable consequences of breaks include substitution of groups of base pairs, rearrangements of groups of base pairs yielding inversions and terminal translocations, and duplications and insertions of one or more base pairs. Such rearrangements have thus far been observed only at grosser levels of chromosome structure than the gene itself.

These chromosomal aberrations are caused by many agents including some which will not induce mutations in *Neurospora* or *Drosophila*. It is not known whether the breaks that cause them are in the backbone of the polynucleotide chain of DNA or whether they involve separation at sites in between the DNA molecules in the chromosome. But they may resemble intragenic point mutations because they may often be accompanied by position effects. In *Drosophila*, and presumably in other organisms, genes confronted with a new chromosomal environment often fail to act, or act differently, or are unstable. When they are returned to the original chromosomal environment, they behave as they originally did, demonstrating that the kind of mutation that involves a new base order has not occurred within them. This points up the suprastructural influences which pervade the genotype, and we may ask whether some unstable genes may not be the expression of chromosomal rearrangements. McClintock has shown that in maize there are mutagenic factors that may move from site to site on a chromosome and from one chromosome to another. When they do so, they change the action of neighboring genes and may even break the chromosome. This calls to mind epi-

somes such as the prophages of bacteria and especially their relative, the *F* factor, which may insert itself in different places on the bacterial chromosome, which it breaks.

In practice, it is often difficult to distinguish the position effects of chromosomal rearrangements from point mutations, as Stadler, the eminent student of mutation in maize, has pointed out. It is a matter of historical interest that the Dutch botanist, De Vries, who was the first serious student of mutation, actually investigated a result of chromosome rearrangement, rather than what we would now call mutation. We have seen how the more sensitive recombination techniques of microbial genetics have recently permitted studies to be made of alterations within the fine structure of genes. Thus in some cases, it is clear that point mutations and not the position effects of chromosomal rearrangements are being investigated.

Still other chromosomal modifications resemble point mutations. In diploid organisms, as we have also seen, it is often necessary to distinguish mutation from certain exchanges occurring between homologous chromosomes. Some "mutations" may actually be due to miscopying under the influence of another allele. Or they may be due to unequal crossing over.

MUTATIONS INDUCED BY RADIATION

High energy radiations are among the best agents for the production of mutations not restricted to sites within the gene. X-rays, for example, frequently induce chromosome breaks. They act on matter by knocking electrons out of the orbits of atoms that absorb their quanta. This results in positively charged particles and free electrons which move at high speed. The latter, in turn, knock other electrons free; eventually when their high energy has been dissipated, the free electrons attach to other atoms. The positively and negatively charged particles which result from these processes are arranged in an ionization track. Each ionization causes a chemical alteration of the charged molecule which becomes chemically exceedingly reactive. The quantitative relationship existing between X-rays and mutation fits this physical picture well and has given rise to what is called the target theory. This theory assumes that the gene is a target and that, when a sufficient number of ionizations (referred to as hits) occur within it, a mutation results. Figure 8.10 shows a curve relating X-ray dose to the number of mutants induced in a large population. Its linearity indicates that one hit is sufficient

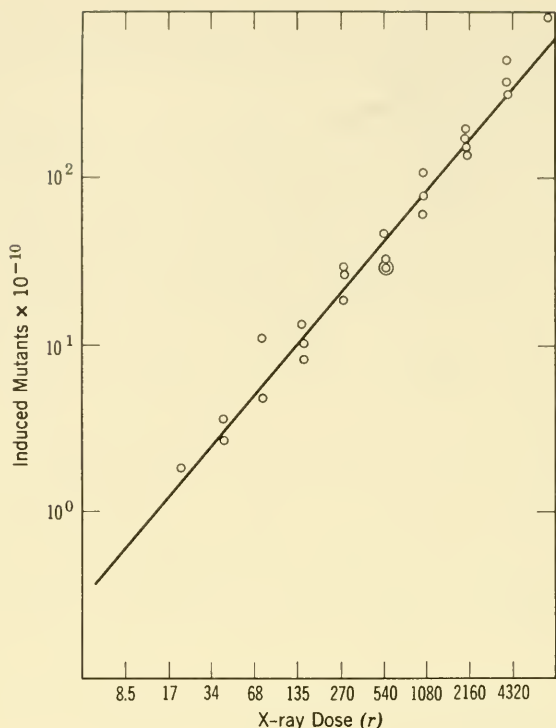


FIGURE 8.10. Number of *met-2*⁺ mutants induced in *E. coli met-2*⁻ by increasingly large doses of X-rays (after Demerec and Sams, in: Buzzati-Traverso, 1960, *Immediate and Low Level Effects of Ionizing Radiations*, p. 283, London, Taylor and Francis, Ltd.).

for mutation because for every aliquot of dose the same number of mutations are induced, according to the equation:

$$M = kD \quad (8.3)$$

where M is the number of induced mutants, k is some dose-independent constant describing the sensitivity of the target, and D is the dose of radiation. Were two hits in one target or one hit in two targets required, the curve would be exponential, the first dose yielding preparatory hits and later doses proportionately more hits within targets already hit once.

That the whole cell is not the target particle is indicated by a calculation of the target size. This is done on the assumption that a larger target would be hit more often than a smaller one by radiation which, like X-rays, is of known density and randomly distributed in space. It

turns out that the target is always considerably smaller than the whole cell, and would seem, therefore, to be some cell particle, perhaps the gene. A number of other quantitative observations fit the target theory; for example, the absence of an observable threshold of dose, independence of the wave length and intensity of the radiation employed, and a cumulative effect even when doses are intermittent. The theory contains assumptions, however, which have not been subjected to a sufficient number of independent tests to warrant unreserved confidence. Furthermore, there is evidence that X-rays may, in part, act indirectly.

Some mutations are lethal and prevent the cell from growing in a given environment. A mutation lethal in one environment may be viable in another. For example, a mutation inducing a requirement for leucine is lethal when that amino acid is absent, but viable when it is present. Other lethals are caused by defects which cannot be repaired, perhaps, in some cases because they concern the synthesis of essential and non-diffusible protein molecules. Then again, many cells may be killed by radiation because of the inactivation of cell structures other than chromosomal genes. If one or another of these processes were responsible for most of the cell deaths resulting from irradiation, then its response to dose could be measured. Often, after exposure to different doses of X-rays, one-hit killing curves are obtained, as in the case with the induction of mutants. But because it is not possible to measure small numbers of dead cells among large numbers of survivors, heavy doses must be employed and the number of survivors decreases significantly with each unit of dose. Nonetheless, if each unit of dose kills the same *fraction* of survivors, then, when the log of the fraction surviving is plotted against dose, a linear killing curve is obtained. An example is shown in Figure 8.11, which follows the relation:

$$S = \frac{N}{N_0} = e^{-kD} \quad (8.4)$$

where S is the fraction surviving, e is the constant 2.718, N is the number surviving after dose D , N_0 is the initial number, and k is a measure of the sensitivity of the cellular unit being inactivated. When two or more units need be inactivated for death to occur, the survival of either one resulting in viability of the cell, another relation, also shown in Figure 8.11, is obtained. It follows the equation:

$$S = 1 - (1 - e^{-kD})^n \quad (8.5)$$

where n is the number of units. Upon the application of low doses, most cells still have at least one surviving unit, but with larger doses even these become inactivated. Eventually, the only cells surviving are those

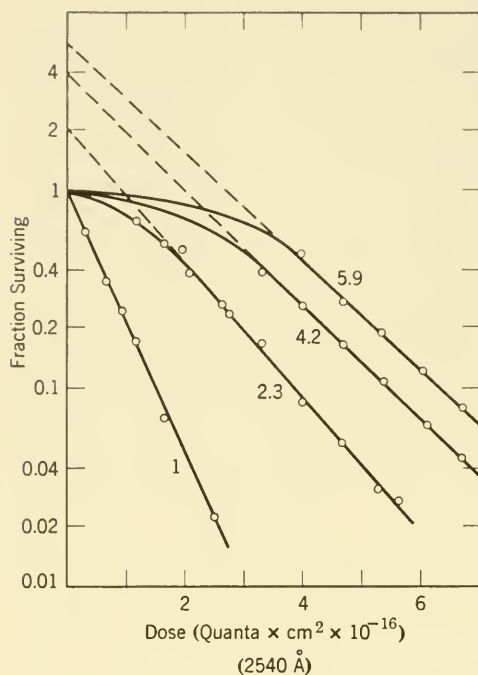


FIGURE 8.11. Killing curves for the asexual spores of *Neurospora* with one or more sensitive units, the inactivation of all of which leads to death. The numbers on the curves indicate the average number of nuclei per spore in the population irradiated (from Norman, 1954, *J. Cell. Comp. Physiol.*, 44:1).

with only one unit left. Thus the curve initially has a shoulder, but eventually a straight line is obtained. The slope of this line is k . It may be greater in the case of the curve for uninucleate cells for a variety of good reasons which are irrelevant here. Whatever the final slope of the multihit curve, if it is extrapolated to the ordinate, the number of units, n , is estimated, because equation 8.5, at high doses, simplifies to:

$$\ln S = \ln n - kD \quad (8.6)$$

In some cases, as in the present example, the number of units corresponds to the number of nuclei.

Chromosomal aberrations, in contrast with gene mutations, do not increase in direct proportion to X-ray dosage but rather increase exponentially (Figure 8.12). Moreover, their number is proportional to the intensity of the radiation and more are produced by continuous than by intermittent treatment. Presumably the breaks themselves are proportional to dose, but after low doses there are not enough breaks to make two in one chromosome or in one nucleus a probable event. As dose increases, however, the number of breaks increases and aberrant fusions become more and more likely. If the intensity is weak or the dose is

intermittent, there is a greater chance that the break will heal in the original configuration before a second break occurs that would allow an aberration. X-ray-induced chromosomal aberrations are observed more frequently at high oxygen tensions, which has led to the belief that X-rays act in an indirect way. Oxygen is important in the formation of H_2O_2 and HO_2 in irradiated water, and it may be these products which induce the breaks. Yet it is still possible to contend that oxygen influences the way in which breaks rejoin; probably it acts in both ways. Many chemicals will also induce chromosome breaks but ultraviolet light induces relatively few. As a matter of fact, UV-induced mutations resemble spontaneous mutations more than do those induced by X-rays.

Ultraviolet radiation is not ionizing; it consists of quanta with less energy and longer wave lengths than X-rays. Substances such as nucleic

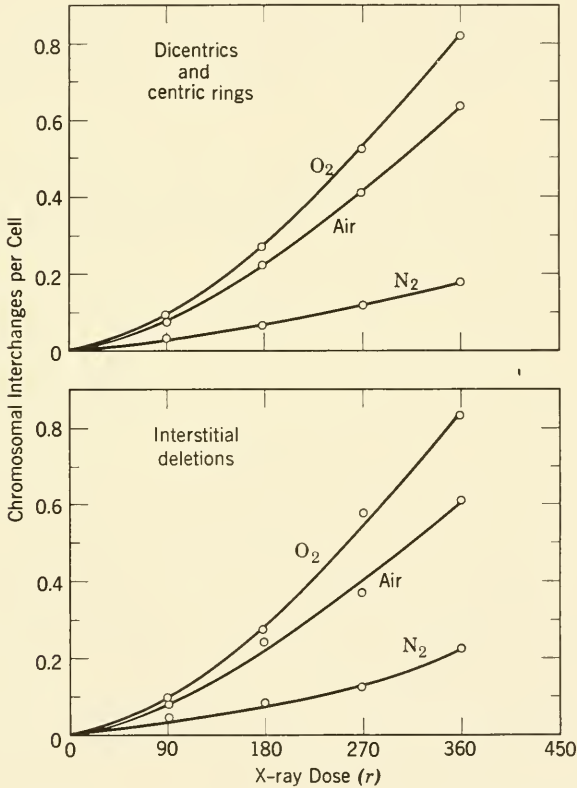


FIGURE 8.12. Relation of chromosomal aberrations in the microspores of *Tradescantia*, to X-ray dosage (from Giles and Riley, 1949, *Proc. Natl. Acad. Sci. Wash.*, **35**:640).

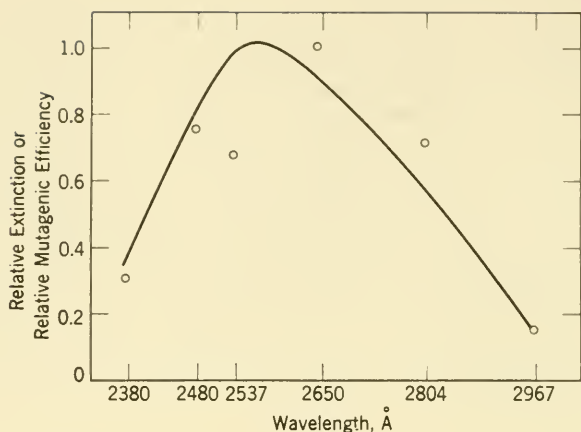


FIGURE 8.13. The absorption spectrum of DNA compared with the action spectrum of mutants induced by ultraviolet light. The points are for mutation in *E. coli* from *str-d* to *str-s*, and the curve is drawn for calf thymus DNA (from Hollaender and Zelle, 1954, *First Intern. Photobiological Congress*, Wageningen, Netherlands, H. Veenman and Zonen, p. 128).

acids absorb the different wave lengths to different extents and, upon doing so, enter an excited state. The absorption spectrum of DNA is shown in Figure 8.13. Superimposed on it is a curve for the number of mutations induced by the same wave lengths. This is called the *action spectrum* of mutation. The similarity suggests that absorption of ultraviolet light by DNA is the cause of mutation. This finding also fits the target theory well, but, again, there is reason to suspend judgment regarding its validity. First of all, a linear relationship between ultraviolet dose and number of induced mutants is not found (Figure 8.14). Furthermore, there is some biochemical evidence involving pre- and post-treatments of the irradiated cells which suggest that the effect of ultraviolet light is not direct. Other substances than DNA, for example RNA and the purines and pyrimidines themselves, have essentially the same absorption spectra. It could be that the initial absorption of the ultraviolet quanta occurred in these substances which exist in the cell outside of the gene. In a chemically modified form they might be incorporated in the replicating DNA and give rise to the mutation observed. If this were the case, the target would be one of these substances rather than the gene itself. In support of this idea is the fact that pretreatment of bacteria with purines and pyrimidines or their ribosides increases the number of mutations induced by subsequent treatment with ultraviolet light. On the other hand, the indirectness men-

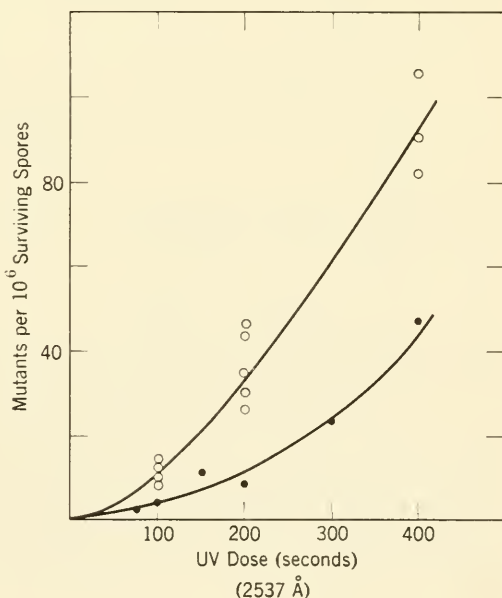
tioned above could be in time only, not in space, so that the gene, upon absorbing a quantum of ultraviolet light, might mutate after an interval; whether it did or not might depend upon the condition of the cell both before and after radiation. As a matter of fact, we must consider both mechanisms possible.

THE METABOLIC CONTROL OF MUTATION

As an example of the indirect action of radiation, we have the observation that the effect of ultraviolet light can be reversed by visible light (*photoreactivation*). For every dose of ultraviolet light which is followed by light reactivation and thus results in a certain number of survivors, a lower ultraviolet dose can be found which, in the absence of light reactivation, yields the same number of survivors. This is called the principle of *dose reduction* and is illustrated in Figure 8.15. The action spectrum of photoreactivation has not led to a discovery of the cellular component that absorbs the visible light, but it is known that the visible light must be applied before DNA synthesis begins. This polynucleotide requires for its synthesis other macromolecules such as protein.

Some of the evidence for this conclusion comes from a study of bacteriophage. After bacteria are infected by phage there is, as Figure 8.16

FIGURE 8.14. Number of ino^+ mutants induced in uninucleate asexual spores of ino^- *Neurospora* by increasingly large doses of ultraviolet light. The data were collected for two different ino^- strains (from Giles, 1951, C. S. H. Symp. Quant. Biol., 16:283).



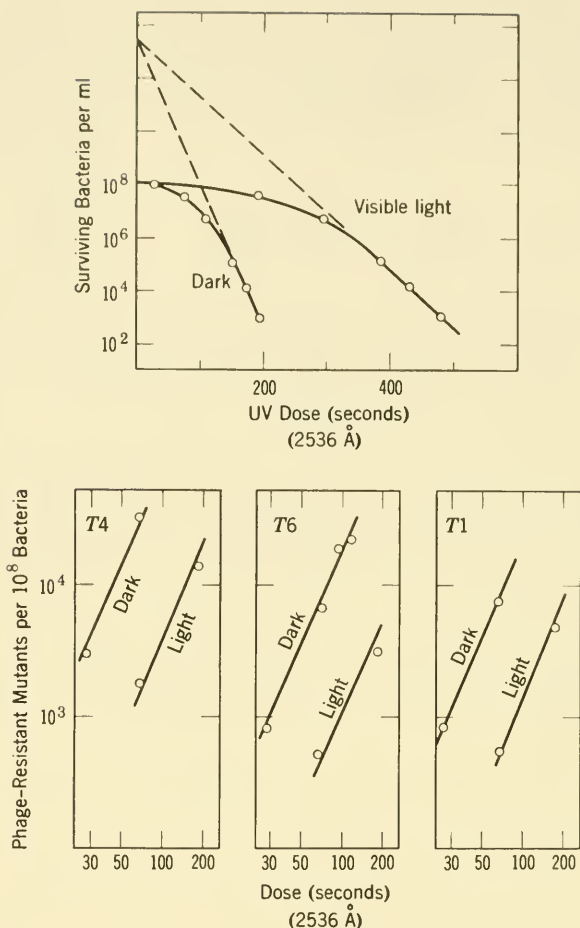


FIGURE 8.15. Reversal of the effects of ultraviolet light by visible light in *E. coli* (from Novick and Szilard, 1949, *Proc. Natl. Acad. Sci. Wash.*, **35**:591).

shows, the immediate synthesis of a new protein; a few minutes later phage DNA begins to increase in amount. If chloramphenicol is present at the time of infection, neither compound is synthesized until the chloramphenicol is removed. But if it is added 5 minutes after infection, when protein synthesis has already begun, then DNA formation will take place even though protein synthesis does not continue. Some protein synthesis seems to be a prerequisite for DNA synthesis. This protein has been shown by isotope tracer experiments not to be a precursor of

the protein in the mature phage. It is known to consist of many enzymes, some of which are directly involved in the polymerization of DNA.

The requirement of protein for DNA synthesis is not restricted to cases such as phage development where the DNA is of a new sort not previously synthesized by the bacterium. The synthesis of any DNA seems to require the synthesis of a protein peculiar to it, either concomitantly, or preceding it. When thymineless-histidineless (*thy⁻ his⁻*) bacteria are starved for thymine but supplied with histidine, essentially no DNA but a good amount of protein is synthesized. Upon transfer of such thymine-starved bacteria into a medium containing thymine, but now no histidine, protein synthesis ceases but abundant DNA synthesis begins (Figure 8.17). Was the previous synthesis of protein required for the formation of DNA? Prevention of the initial protein synthesis during thymine starvation by chloramphenicol shows that it was. For, when these bacteria are transferred to medium with thymine but with no histidine or chloramphenicol, protein synthesis still does not take place. Furthermore, the vigorous synthesis of DNA observed in the first instance does not take place; only a minimal synthesis characteristic of cells that have not been starved for thymine is observed. The new synthesis of protein during thymine starvation was required for the excess DNA that formed when thymine was subsequently provided.

Some studies with ultraviolet light similarly implicate protein in the process of DNA replication and in the process of mutation as well.

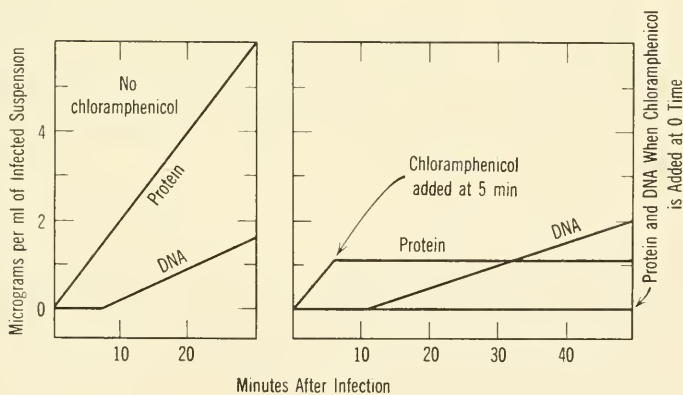


FIGURE 8.16. A representation of the effect of chloramphenicol on the synthesis of protein and DNA by phage-infected bacteria (after Tomizawa and Sunakawa, 1956, *J. Gen. Physiol.*, 39:553).

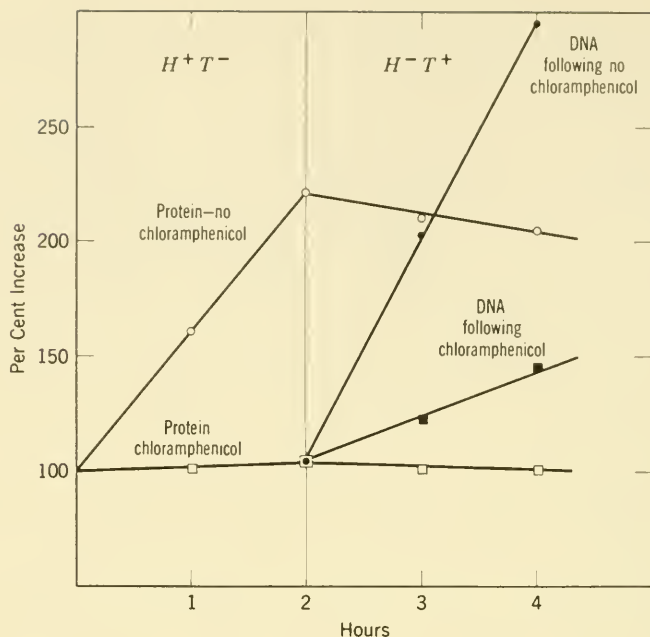


FIGURE 8.17. The role of protein in DNA synthesis. The left half of the figure shows the change in the protein content of *E. coli* $thy^- his^-$ starved for thymine but provided with histidine ($H^+ T^-$) in the presence or absence of chloramphenicol. The DNA in these cells does not increase by more than 5 per cent. During the 2 hours involved, the viable cell number remains constant; only afterwards does the thymineless death characteristic of thy^- bacteria set in. The right half of the figure shows the behavior of protein and DNA when the cells are washed and suspended in medium with thymine but devoid of histidine ($H^- T^+$). Here again they do not die for at least two hours. In the absence of histidine, no protein synthesis occurs. But DNA increases to an extent dependent upon whether the previous starvation for thymine took place in the absence or presence of chloramphenicol, without or with the synthesis of protein (from Nakada, 1960, *Biophys. Biochim. Acta.*, 44:241).

When bacteria are irradiated with mild doses of ultraviolet light, DNA synthesis is temporarily arrested and the length of the delay is a function of the dose used. Protein synthesis can be blocked at the same time if specific inhibitors such as chloramphenicol are added after irradiation or if amino acid auxotrophs are starved for their requirement. In these cases, DNA formation does not begin until protein synthesis is allowed again. Witkin has shown that some UV-induced mutations require the presence of amino acids for their realization. Even when amino acids

are present, the inhibitor of protein synthesis, chloramphenicol, prevents their effect. Furthermore, the formation of abnormal protein containing amino acid analogues decreases the mutant yield. Consequently, protein synthesis seems a prerequisite for DNA formation and, as a result, for mutation. These effects on mutation can be observed only during the initial part of the first postirradiation division whose length can be increased by the dose of ultraviolet light and by nonmutagenic concentrations of caffeine, which inhibit DNA synthesis. After this division the primary effect of ultraviolet light on the cell, perhaps a pool of modified DNA precursors, has been dissipated.

Protein synthesis is not the only process that is prerequisite for DNA formation and mutation; its concomitant, RNA synthesis, is also required. A turnover of RNA precedes DNA synthesis in phage-infected bacteria. Furthermore, the role of RNA can be shown by the use of inhibitors and the starvation of uracilless auxotrophs after irradiation. In cells so treated, DNA synthesis is not restored until RNA synthesis is permitted again.

But what can DNA synthesis do to establish the mutation? Ultraviolet light, like other radiations, induces chemical modifications in substances that absorb it. Some, including water and amino acids, are converted into highly reactive free radicals and peroxides which are mutagenic, but many of these products are unstable or are decomposed by the cell before they can act. When adenine, guanine, cytosine, uracil, or their ribosides are given to bacteria just before irradiation, the yield of mutants is increased. When these substances are irradiated by themselves, they form unstable products. It may be that the immediate effect of UV-irradiation is the modification of nucleic acid precursors. The synthesis of protein and RNA may be necessary for the incorporation of such modified precursors into DNA and for its subsequent replication which completes the change of genotype.

Thus we should think of mutation as quite different from the spontaneous disintegrations of radioactive atoms which obey monomolecular kinetics and are independent of environmental conditions. This is true also of spontaneous mutations; not only are they reversible but also in other ways their properties are those of biochemical processes. For example, spontaneous mutations usually have a Q_{10} of 2 or 3, as is shown in Figure 8.18. The fact that spontaneous mutations do not occur at temperatures as low as 0° (although the cells survive) indicates that they are not, for the most part, induced by local molecular agitation which, with a low frequency proportional to temperature, would involve energies high enough to induce a rare chemical change in the gene. If

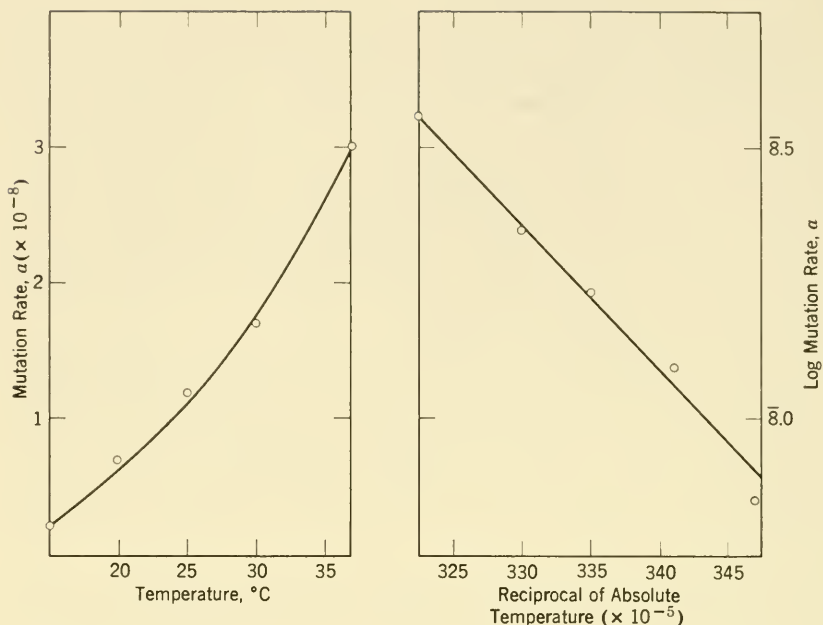


FIGURE 8.18. The temperature relation of mutation from his^- to his^+ in *E. coli* (from Ryan and Kiritani, 1959, *J. Gen. Microbiol.*, **20**:644).

the latter were so, the threshold of the temperature relation, instead of being above zero, should be absolute zero (-273°C), at which temperature molecular agitation is nil. The existence of a temperature relation does not entirely preclude the possibility that natural radiation is the eventual cause of mutation because its effects are indirect, acting through intermediate chemical reactions. Actually, this possibility is excluded for most mutations by quantitative comparisons of the frequency of radiation-induced mutation with doses of radiation measured in the environment. Less than 0.1 per cent of mutation in *Drosophila* can be accounted for in this way, although in man, whose life span is long, a fair proportion of all mutations may be induced by natural radiation. We may conclude that some extragenic chemical processes limit the rate at which spontaneous mutation occurs. The same can be said for induced mutation. The chemical processes inferred may produce intracellular mutagens or they may be only those involved in the synthesis of the purine and pyrimidine bases, of the nucleosides and nucleotides, and of the polynucleotides themselves.

DIRECTED MUTATION

Students of mutation have long hoped to understand the process sufficiently well to bring it under control. Although all known mutagens are effective against many genes, it has been found that most mutants respond to some mutagens and not to others; usually the differences are only quantitative. Chemical mutagens appear to act with different degrees of directness that have not yet been sufficiently analyzed; nonetheless, the best examples of specificity come from studies of chemically induced back mutation in a double auxotroph of *Neurospora*. Here both genes studied are in the same nucleus and, therefore, are under conditions as similar as can be experimentally achieved. As is shown in Table 8.2, the inositolless locus is most responsive to ethylmethane sulfonate and least responsive to bromethyl methane sulfonate. In the case of the adenineless locus, the situation is almost the reverse. Such specific re-

TABLE 8.2
The Relative Specificity of Six Mutagens on the
Double Inositolless-Adenineless Mutant of *Neurospora*

(From Westergaard, 1957, in Wolstenholme and O'Connor, *Ciba Found. Symp. on Drug Resistance in Microorganisms*, Boston: Little, Brown & Co., p. 280; and Kolmark, 1956, *C. R. Trav. Lab. Carlsberg, Ser. Physiol.*, **26**:205; and Westergaard, 1960, *Chemische Mutagenese, Abh. deut. Akad. Wiss. Berlin, Kl. Med.*, No. 1, 1960, p. 30)

Mutagen	Mutation per 10 ⁶ Asexual Spores <i>inos</i> ⁺	<i>ad</i> ⁺	Proportion <i>ad</i> ⁺ / <i>inos</i> ⁺
Ethylmethane sulfonate	11.3	17.4	1.5
Diethyl sulfate	4.3	16.8	3.9
Dimethyl sulfate	3.4	64.0	19.0
Chloroethyl methane sulfonate	0.3	51.0	190.0
Dipropyl sulfate	0.2	13.6	68.0
Diepoxybutane	0.2	89.0	445.0
Bromoethyl methane sulfonate	0.04	152.0	3800.0
Ultraviolet light	7.1	3.5	0.5
X-rays, 10 ⁵ r	0.2	3.2	16.0
Spontaneous	0.02	0.2	10.0

sponses are not found when forward mutation from the wild-type alleles is studied, presumably because so many modifications within each of them will yield an auxotroph. Each auxotroph arising by point mutation carries, on the contrary, a specific modification at a specific site, and only when this is restored to the wild-type condition is there a back mutation. Although the average base composition of genes may turn out to be similar, we have not yet the necessary evidence to decide whether all genes are of similar size. Clearly the total number of nucleotide pairs should have something to do with stability and apparently so does the particular pattern of nucleotides within the gene, for a particular site does not mutate at a rate independent of neighboring nucleotides. The base sequences of different genes may soon be known; we must hope that, in the course of their determination, something will be learned which will enable us to circumvent the common denominators which make them so similar in stability. Otherwise the elective mutation of chromosomal genes seems a hopeless ambition.

It is otherwise with extra-chromosomal genes; mutants for some of these have already been induced electively. Hereditary respiratory deficiency can be induced at will in yeast by the use of acridine dyes; the same is true for the conversion of F^+ to F^- *E. coli*, and for the loss of the basal granule in trypanosomes. By treatment with streptomycin, *Euglena* can be permanently freed of its chloroplasts. But these mutations may involve only the loss of an extra-chromosomal particle by destruction or the inhibition of its replication. Chapter 9 will deal with this question.

BIBLIOGRAPHY

- Freese, E., 1959. On the molecular explanation of spontaneous and induced mutations. *Brookhaven Symp. Biology*, **12**:63-75.
- Freese, E., 1959. The difference between spontaneous and base-analogue induced mutations of phage T4. *Proc. Natl. Acad. Sci. Wash.*, **45**:622-633.
- Freese, E., 1959. The specific mutagenic effect of base analogues on phage T4. *J. Molecular Biol.*, **1**:87-105.
- Giles, N. H., 1959. Mutations at specific loci in *Neurospora*. *Proc. X Inter. Congress of Genetics*, Montreal, 1958, **1**:261-279.
- Hollaender, A., 1954. *Radiation Biology*. New York, McGraw-Hill, 3 vols.
- Lea, D. E., 1955. *Action of Radiations on Living Cells*. Cambridge, The University Press, 2nd ed., 416 pp.
- Martin, J. H. (ed.), 1959. *Radiation Biology*. Proc. 2nd Australian Conference, Melbourne, 1958. New York, Academic Press, Inc., 305 pp.

- Miner, R. W. (ed.), 1955. Ionizing radiation and the cell. *Am. N. Y. Acad. Sci.*, **59**:467-664.
- Novick, A., 1956. Mutagens and antimutagens. *Brookhaven Symp. Biology*, **8**:201-215.
- Witkin, E. M., 1956. Time, temperature and protein synthesis; a study of ultraviolet-induced mutation in bacteria. *C. S. H. Symp. Quant. Biol.*, **21**:123-140.
- Witkin, E. M., 1959. Post-irradiation metabolism and the timing of ultraviolet-induced mutations in bacteria. *Proc. X Inter. Congress of Genetics*, Montreal, 1958, **1**:280-299.

9 Nonchromosomal genes

Are Mendelian genes the sole hereditary determinants of the organism? This question was first posed in the period 1900–1910, following the rediscovery of Mendelian inheritance in 1900. Because the precision and simplicity of unit factor segregation seemed so much at variance with the obvious complexity of biological systems, many investigators accepted the evidence of Mendelian inheritance with great reluctance. Some felt that the described instances were exceptional, that the attempt to generalize “Mendelian laws” was pushing the data too far, and that other mechanisms of heredity must also exist.

Most impressive was the skepticism of T. H. Morgan, expressed just a short time before he left his embryological research for genetics. In an address to the American Breeders Association in 1909, he said:

In the modern interpretation of Mendelism, facts are being transformed into factors at a rapid rate. If one factor will not explain the facts, then two are invoked; if two prove insufficient, three will sometimes work. The superior jugglery sometimes necessary to account for the results may blind us, if taken too naively, to the common-place that the results are often so excellently explained because the explanation was invented to explain them.

Within a year Morgan began to work with *Drosophila*, and soon a wealth of spontaneous mutants were isolated, providing magnificent material for demonstrating the generality of Mendelian segregation and leading to Morgan’s formulation of the chromosomal theory of heredity. The wide range of phenotypes altered by mutation, and the rarity of aberrant segregation, led many investigators to conclude that chromosomal factors could well account for the total heredity of the cell.

However, also in 1909, the first paper on non-Mendelian heredity was published. Its author, the German, Carl Correns, was one of the three

rediscoverers of Mendelism. It was his view that more than one system of heredity might well exist, and that both Mendelian and non-Mendelian patterns of inheritance merited investigation. In fact, his own researches continued to include inquiries into both systems throughout his life. Subsequently, other leading geneticists including Baur, Winge, von Wettstein, and Renner in Europe, and Rhoades in the United States carried out extensive studies of non-Mendelian heredity in higher plants and firmly established the widespread occurrence of this phenomenon. Rhoades' analyses of the non-Mendelian inheritance of pollen sterility and of damaged chloroplasts in maize, to be taken up later, are unambiguous demonstrations of the existence of stable permanent genetic determinants which do not segregate as chromosomal elements.

A different impetus for further investigation of nonchromosomal heredity has come from cytologists, remarking on the regularities and precision of the organization of cytoplasmic structures, and also upon the observed multiplication of cytoplasmic organelles by dividing, rather than by forming *de novo* out of undifferentiated cytoplasm. This mode of origin of organelles from pre-existing organelles might indicate the existence of some kind of replication. As early as 1908, Meves proposed that mitochondria might carry their own hereditary determinants, but no genetical system was available to test the hypothesis.

Recent advances in our understanding of nonchromosomal heredity have come principally from work with microorganisms. The purpose of this chapter will be to describe the methods employed for analysis, with a few examples, and to summarize the present level of knowledge in this field.

DEFINITIONS AND CRITERIA

In Chapter 1, we defined a hereditary determinant as a unit of inheritance essential for the appearance of a particular character. We are now in a position to refine this concept. On the basis of much indirect evidence, we have come to associate heredity with particular macromolecular configurations. As a general definition, it is proposed that heredity consists of the conservation of specificity during replication and, in this sense, that a hereditary determinant is a genetically functional unit which is replicated with conservation of specificity.

Detection of hereditary determinants, on this formulation, requires a method to identify genetically functional units, and a method to study their replication. With respect to chromosomal factors, the classical method of identification has been recombination analysis, including the

neoclassical methods of transformation and transduction. Nonchromosomal elements have been identified by their aberrant segregation patterns in meiosis, which make them unmapable in any chromosomal linkage group, and often by their segregation in mitosis under conditions where chromosome segregation is not occurring.

The study of replication is potentially a powerful tool in the analysis of heredity. The term replication is employed in modern genetics to mean the origin of a new unit by copying from a pre-existing unit of the same kind, either directly in one step or indirectly with the intervention of complementary intermediates. The only cell elements which need be considered as possible carriers of heredity are those which do replicate, and do so, furthermore, with conservation of specificity. As yet, the only biological system smaller than the whole cell in which the process of replication has been shown to occur is the DNA.

Other species of molecules to be considered as possible carriers of heredity include RNA and proteins. With neither class of compounds has the mode of synthesis been fully established (see Chapter 11). Protein specificity is probably determined by a coding device utilizing RNA, and does not seem to require a pre-existing protein molecule of the same kind for its synthesis. On the other hand, studies of protein synthesis have been limited largely to a few soluble proteins, and much less is known about the synthesis of the structural proteins, some of which like the cytochromes are enzymatically very active.

Very little is known as yet about RNA synthesis (see Chapter 11), but again there are different types of RNA and some of them may be replicated. The existence of viruses, in which the hereditary information is carried by the RNA, requires that *that* RNA at least be replicated with conservation of its specificity. Since a replication mechanism must exist for viral RNA, perhaps the same mechanism is employed for some cellular RNA's as well.

If it were possible to detect molecular replication directly, a method would thereby be available to screen for hereditary determinants on that criterion alone. Since no such methods are yet available, we must turn to the classical methodology of genetics for evidence of nonchromosomal heredity.

Nonchromosomal determinants have been identified primarily by their nonsegregation at meiosis. In such instances, crosses between contrasting parents give rise to progeny exclusively of one parental type. An example is the inheritance of streptomycin resistance in *Chlamydomonas* (see Chapter 3 for its life cycle).

The wild-type cells are streptomycin-sensitive (*ss*), and by treating a large population of cells with the drug, it is possible to isolate resistant

mutants. Two classes of streptomycin-resistant (*sr*) mutant strains have been obtained: those which show typical Mendelian inheritance in crosses, and those which do not. As an example of the latter class, Figure 9.1 shows a diagram of a cross between a resistant strain, *sr-500*, and a sensitive strain, *ss*.

When *sr-500* cells of mating-type plus (mt^+) are crossed to any *ss* strain of mating-type minus (mt^-), all progeny are found to be uniformly

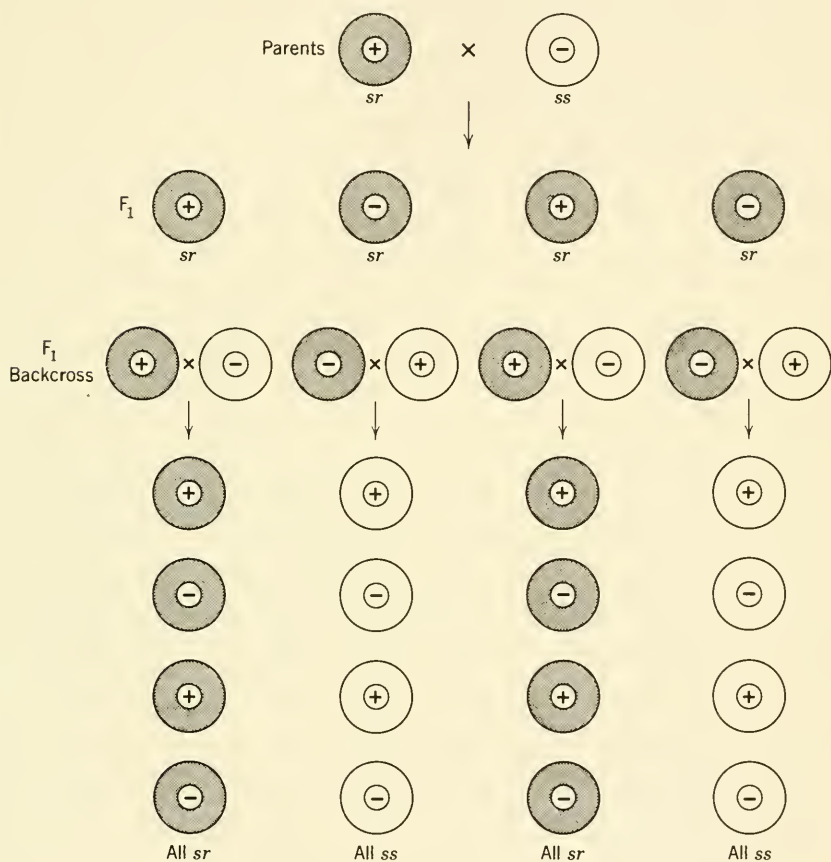


FIGURE 9.1. Nonchromosomal inheritance in *Chlamydomonas*. Plus and minus signs refer to mating type which is inherited as a chromosomal gene difference. The cross $sr\ mt^+ \times ss\ mt^-$ gives rise exclusively to *sr* offspring which segregate 2:2 for mating type and other chromosomal genes. F_1 clones of plus mating type backcrossed to *ss* mt^- produce all *sr* offspring (4:0) but F_1 clones of minus mating type backcrossed to *ss* mt^+ produce only *ss* progeny (0:4) (after Sager, 1954, *Proc. Natl. Acad. Sci. Wash.*, **40**:356).

streptomycin-resistant to the same high level as the parental *sr-500* strain, even after years of continuous cultivation in the absence of streptomycin. The possibility that this result derives from the presence of many different chromosomal genes, each exerting an effect on resistance (i.e., multifactorial inheritance), was ruled out by four successive generations of back-crossing of resistant progeny to the sensitive parent of *mt*⁻. The same results were obtained in each generation: there was no segregation of sensitivity among the progeny. All were uniformly resistant, although chromosomal genes segregated normally in the expected 1:1 ratio.

In the reciprocal cross, *sr-500 mt*⁻ × *ss mt*⁺, all progeny were found to be uniformly streptomycin-sensitive, and in subsequent crosses the resistance factor did not reappear. The difference on the part of the two mating types in their ability to transmit the *sr-500* factor was not correlated with any phenotypic differences in resistance level or stability. One technique for studying nonchromosomal determinants is based on the idea that they may not be well integrated in cellular division, and that, by growing cells at maximal division rates, they might be diluted out; or under difficult growing conditions they might be lost. To test these possibilities in the *sr-500* system of *Chlamydomonas*, vegetative strains of both mating types were grown under a wide variety of nutritional conditions at rates from a 5- to a 48-hour generation time. Neither these conditions nor heat shock, cold treatment, or UV-radiation gave rise to any streptomycin-sensitive cells. In fact, the stability of the *sr-500* property in the absence of streptomycin is impressive. No reversion to sensitivity has been found over a several-year period.

These results indicate that the *sr-500* determinant is well integrated into some replicating mechanism of the cell; and that it must be considered a stable, permanent constituent of the hereditary material, ranking with a chromosomal gene. The results of crosses demonstrate that the *sr-500* factor is nonchromosomal, on the basis of its nonsegregation in meiosis and its consequent nonmapability. The uniparental transmission of *sr-500* by parents of mating-type plus but not of mating-type minus resembles maternal inheritance, as for example in the *poky* mutant of *Neurospora*.

In *Neurospora*, a slow-growing strain called *poky* was crossed with wild type, and was found to exhibit nonsegregation in meiosis, uniparental inheritance, and non-mapability, as in the *Chlamydomonas* system. In *Neurospora* (see Figure 3.2), either mating type can produce fruiting bodies which contribute not only a haploid nucleus but also the cytoplasm to the zygote. The fruiting body can be fertilized by conidia of the opposite mating type which contribute the other haploid

nucleus and probably no cytoplasm. Thus, reciprocal crosses can be made with this material.

As shown in Figure 9.2, the poky trait is transmitted to all progeny when carried by the maternal parent, and to none when carried by the conidial parent. The difference in reciprocal crosses may result from the fact that conidia transmit little or no cytoplasm, but there is no direct evidence on this point.

The poky mutant and a similar strain subsequently isolated (*mi-3*) have been further investigated with respect to their stability and to the biochemical basis of the poky phenotype. There has been no change in

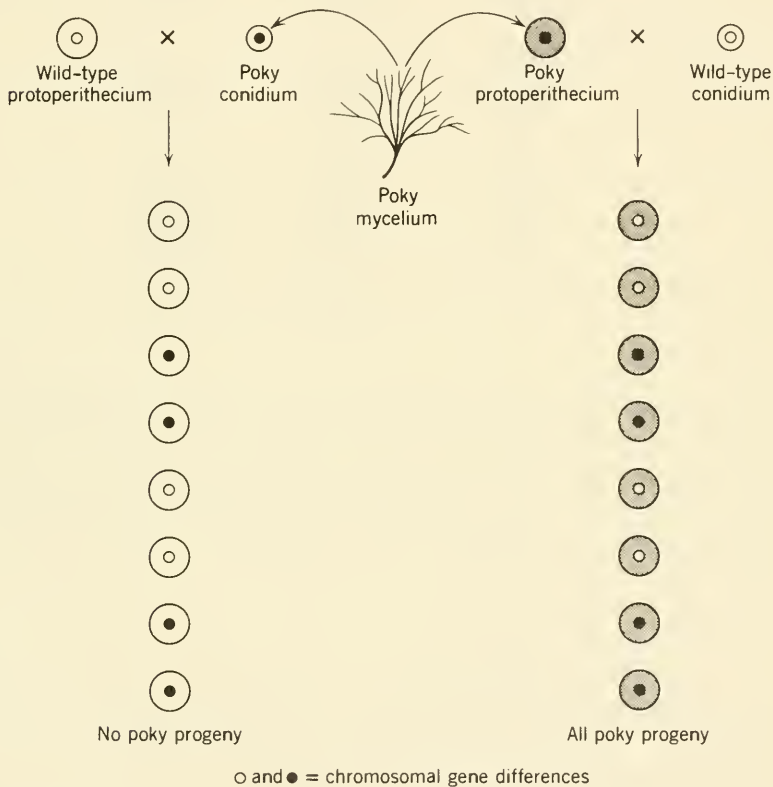


FIGURE 9.2. Nonchromosomal inheritance in *Neurospora*. From the mycelium of a poky strain, both female (protoperithecia) and male (conidia) gametes are formed, so that reciprocal crosses can be performed with a wild-type strain. When poky is used as female parent, all the progeny are poky, and in the reciprocal cross all the progeny are nonpoky; in both cases, segregation is 1 : 1 for other chromosomal genes segregating in the cross (after Mitchell and Mitchell, 1952, *Proc. Natl. Acad. Sci. Wash.*, **38**:442).

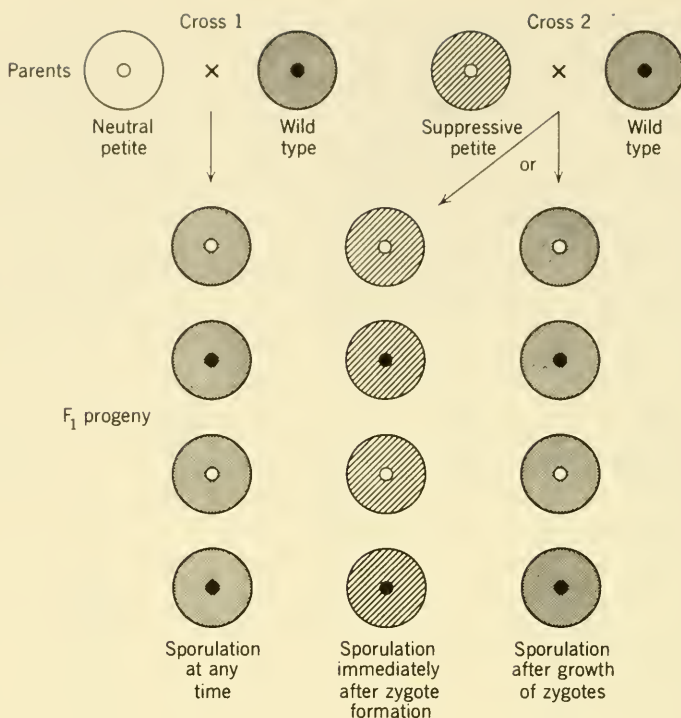


FIGURE 9.3. Nonchromosomal inheritance in yeast. In crosses of wild type with neutral petites, all the progeny are wild type regardless of time of sporulation, but the suppressive petites behave differently. If the zygotes are sporulated immediately, all progeny are petite, but if zygotes are allowed to grow vegetatively for a number of generations and then sporulated, all the progeny are wild type, as in the case of neutral petites. Chromosomal genes segregate 2:2 in all crosses (after Ephrussi, Hottinguer, and Roman, 1955, *Proc. Natl. Acad. Sci. Wash.*, 41:1065).

either the pattern of inheritance or in the phenotype upon repeated transfer, indicating that the hereditary determinant is stable, as in the *Chlamydomonas* system. In the presence of the poky factor, the activities of the cytochrome enzymes are altered, and terminal oxidation is decreased, accounting probably for the slow growth. The primary action of poky is not known, but there is evidence that the structure as well as the functional abilities of the mitochondria have been affected.

Petite strains of yeast have been described which exhibit non-chromosomal inheritance. These strains, which produce tiny colonies on agar, have no cytochrome oxidase activity and ferment glucose in the

presence of oxygen as if they were growing anaerobically. Detailed studies of their respiratory enzymes have shown that those which are of mitochondrial location in normal strains are absent in petite strains, although morphological structures corresponding to mitochondria can be found by electron microscopy.

When petite strains are crossed with normal yeast (Figure 9.3) the petite phenotype does not segregate regularly among the progeny. With strains called neutral petites, all the progeny are normal: the petite factor is not transmitted through the cross. In other strains, called suppressive petites, the petite factor may be transmitted to all progeny or to none, depending upon the precise environmental conditions during germination.

The fact that the transmission of petite can be altered by the environment may eventually shed some light on the mechanism by which non-chromosomal determinants are distributed at meiosis. At present, one may only speculate that a common mechanism of transmission for non-chromosomal determinants at meiosis may exist in all organisms, despite the apparently special feature that uniparental transmission is linked with mating type in some species and not in others.

In summary, it is the absence of segregation in meiosis, the disappearance of one of the two parental phenotypes, and the consequent non-mapability of the hereditary factor which distinguish nonchromosomal inheritance so sharply from that of determinants carried on chromosomes, in the cited examples. The same criteria apply in most other examples of nonchromosomal inheritance, of which there are some hundred well-studied instances in the literature, most of them from plant material. There are also some examples of erratic segregation patterns, as in the *iojap* system in maize, to be discussed below.

Additional criteria which have been employed to distinguish between chromosomal and nonchromosomal genes include the occurrence of segregation during vegetative growth, giving rise to sectors (not associated with somatic recombination), chemical induction of mutation, and infectivity in the sense of transfer of the determinant from cell to cell in transient fusions not involving chromosome transfer.

Operationally, this mode of inheritance can only be referred to as non-chromosomal, although it is often spoken of as cytoplasmic. It should be understood, of course, that many mechanisms can produce aberrant segregation ratios, such as physiological effects of parental cytoplasm, multifactorial determination of phenotype, and various sorts of chromosome aberration. These must all be excluded by suitable experiments before the existence of a nonchromosomal determinant may be properly invoked to account for the data.

The various patterns of nonchromosomal inheritance which have been observed experimentally, such as those found in *Chlamydomonas*, yeast, and *Neurospora*, provide no definitive information about the location or nature of the determinants, or about their replication. The apparently equal distribution of determinants to all progeny could be achieved by: (a) random distribution of a large number of replicates; or (b) synchronized replication and oriented transmission of one or a few replicates.

A nonchromosomal unit factor might exhibit unit factor inheritance, mimicking Mendelian inheritance, but provide no evidence of linkage. In practice, the allelic pairs studied in *Drosophila*, maize, and *Neurospora* have been mapable, but one may wonder whether there may be nonchromosomal units which are indispensable or nonmutable, and therefore undetected. Unit organelles like centrioles might be an example. In *Chlamydomonas*, which contains just one chloroplast per cell, a mutation which blocks chlorophyll synthesis has been found to segregate only at the first division of meiosis. This result may be interpreted either as close linkage to a centromere or as evidence of a nonchromosomal location. No linkage with any other marker has yet been observed.

In the absence of direct identification of any permanent cell constituent as a nonchromosomal gene, a number of hypotheses have been developed to define these units in a testable manner. Before turning to a more detailed consideration of the genetic evidence, it might be well to consider briefly a few of the leading current proposals.

1. The *gene* hypothesis states that nonchromosomal genes are molecular entities with a permanence and stability comparable to that of chromosomal genes. They are replicated with conservation of specificity in a manner integrated with cell division, and their replication does not depend upon their phenotypic expression. In other words, on this hypothesis, nonchromosomal factors rank with chromosomal ones; they are simply genes without chromosomes.

2. The *supertemplate* hypothesis proposes that some structural elements, such as mitochondrial and chloroplast membranes with their constituent macromolecules requiring precise orientation for activity, may be formed by some copying process from a pre-existing surface of the same kind. DNA replication seems to involve ordering in one dimension only, and the determination of protein specificity by arranging amino acids in a proper linear sequence has also been considered as a one-dimensional problem. The supertemplates postulated here might be two-dimensional.

3. The *steady-state* hypothesis proposes that a system of interlocking enzymatic reactions can be set in motion by a particular environmental influence, and have such intrinsic stability as to be perpetuated indefinitely under suitable environmental conditions. A sudden shift in environment may induce a metabolic shift to a new steady state, mimicking a mutational change. Since the degree of susceptibility to environmental stress will vary with the particular system, no general prediction about stability can be made. However, it is just this ill-defined aspect of the hypothesis that weakens it by making it virtually incapable of disproof, except by demonstrating what is the actual mechanism. Since an essential feature of the steady-state system is its regulation by environmental influences, it is likely that stable nonchromosomal determinants are not of this type, in particular those maintained for long periods under diverse environments in a latent state, with their phenotypes unexpressed (e.g., streptomycin resistance in the absence of streptomycin).

4. The *episome* hypothesis of Jacob and Wollman has been proposed only with respect to dispensable functions, those without which an organism can still survive (see Chapter 5). The term was initially applied to prophages and to the *F* factor in *E. coli* which can exist in two alternative states: either integrated with the chromosome and not infectious, or unintegrated in the sense of being unmapable, infectious, and not dividing synchronously with the cell. Episomes should be mapable in the integrated state, and therefore readily distinguishable from nonchromosomal genes. However, if one postulates that the factor always becomes unintegrated in mitosis and meiosis, a most unlikely possibility, then, of course, mapability is lost and only the unintegrated state can be studied.

No factors have been found as yet in other systems which fit the criteria for episomes, but several have been described with some common features. For example, CO₂-sensitivity and sex ratio in *Drosophila*, the killer trait in *Paramecium*, barrage and senescence in *Podospira*, all depend upon the presence of an infectious unintegrated nonchromosomal factor.

The method of choice for distinguishing among these alternatives would be transformation or infection: that is, the establishment of the new determinant in cells of strain *A* by introducing a defined cell constituent from strain *B*. In most systems, this experiment has not succeeded, and only indirect criteria have been available for judging the nature of the nonchromosomal genes. Because of the complexities of the problem and the indirect methods of analysis, diverse systems have been grouped as nonchromosomal. Probably they include examples consistent with

each of the above hypotheses which, of course, are not mutually exclusive (except as applied to individual cases).

We shall review some systems among which may be examples of each type. First it is appropriate to consider what is known about the organization of cell structure, especially in relation to replicating mechanisms other than that for DNA.

A REVIEW OF CELL STRUCTURE

The precision of mitosis and meiosis implies a precise copying mechanism; consequently, observations of these events led some cytologists to predict an essential role for chromosomes in heredity before any supporting evidence was available from genetics. No other cell structures have been found which exhibit similar regularities in pairing and segregation at the level of the light microscope. Therefore, no obvious candidate comes to mind as the carrier of nonchromosomal heredity. On the other hand, many cell organelles can be seen to divide, suggesting the existence of a submicroscopic copying mechanism.

When cell structures are observed with the electron microscope, at the level of their macromolecular architecture, one still does not find clear evidence of replication. However, a basic uniformity of structural organization is found in most or all cell types, involving membranes, particles, and organelles such as mitochondria, so well defined as to merit evaluation as possible autonomous hereditary systems. Furthermore, since RNA may carry hereditary information, evidence on the localization of RNA's within particular cell structures may provide some clues for the identification of nonchromosomal determinants.

In this brief survey we shall consider: (1) the membranous systems of the cytoplasm; (2) the particulate components which contain most of the high molecular weight RNA of the cell, namely, the ribosomes of the cytoplasm and the nucleolus of the nucleus; and (3) organelles such as mitochondria, chloroplasts, and kinetosomes.

Membranous Systems of the Cytoplasm

As the techniques for electron microscopy of sectioned cells developed over the last several years, new membranous systems of the cytoplasm were observed, described, and named. Subsequently, as connections were found between these systems, it became more and more likely that they represented regional differentiations of a single continuous network,

including the nuclear membrane, the plasma membrane, the Golgi membranes, and the endoplasmic reticulum (ER).

The nuclear membrane is a double envelope peppered with perforations which may or may not be fully permeable to the passage of materials between nucleus and cytoplasm. The ER, a network of membrane-bound canals and vesicles, which is profuse in some cell types and scant in others, connects the plasma membrane at its outer limit with the nuclear envelope at the cell center. When synthesis of a secretory protein is in progress, as in the acinar cells of the pancreas, the ER is seen to serve as a channel for passage of newly formed protein to storage vacuoles and, ultimately, to the plasma membrane. Protein synthesis occurs in association with the ribosomes, also called microsomal particles, which in these pancreatic cells are attached in abundance to the membranes.

Both rough (ribosome-loaded) and smooth-surfaced membranes of the ER have been described. The Golgi apparatus, consisting of a characteristic arrangement of densely packed flattened vesicles near the cell center, seems to be a reservoir of membranes, available for transport of cell products or for other purposes. Connections have often been described between smooth-surfaced Golgi membranes and membranes of the ER. The apparent interconvertibility of cell membranes suggests that they possess a common molecular structure, or else reversible flexibility in incorporating or giving up particular structural constituents. Examples of these structures are shown in Plates XI, XII and XIII.

Ribosomes are dense spherical granules about $150 \text{ \AA}$ in diameter, as seen with the electron microscope; they contain most of the cytoplasmic RNA. Found in profusion on the outer surface of the nuclear membrane in most cell types, their concentration varies with cell function in other parts of the cytoplasm. For example, in the acinar cells of the pancreas, during periods of rapid protein synthesis, ribosomes are present in large numbers, located primarily on the membranes of the ER. In dividing cells, for example in the alga *Chlamydomonas*, most of the ribosomes are free in the cytoplasm. In other materials, one finds various combinations of free and bound particles. The significance of this distribution is not known, but it seems likely that the particles are functional in both conditions, whether bound or free. In bacteria, ribosomes are found throughout the cytoplasm in the free form.

Four sizes of ribosomes have been identified in extracts of mammalian tissues, higher plants, yeast, and bacteria. Although the precise sizes vary somewhat with the species, the general picture is the same for all (Plate IV). For example, the predominant particle found in rapidly growing bacteria is identified by its sedimentation rate in the ultra-

centrifuge, as 70S. It consists of two basic units of unequal size and shape, a 30S and a 50S particle; magnesium ions play an important role in holding the 70S particle together. Sometimes two 70S particles aggregate to form one unit which sediments at 100S.

Nucleoli are spherical structures found within the nucleus during interphase; cells may have one or several of them. They break down at metaphase, reappear at telophase, and their reorganization has been shown to be under chromosomal control. In the electron microscope, the nucleolus appears granular, composed of particles about 150 Å in diameter, and without any limiting membrane. A few chemical and cytochemical studies have demonstrated that nucleoli are very dense, probably quite dehydrated, consisting mainly of protein, the major component of which in some organisms is an acidic protein rich in glutamic acid, and 2 to 5 per cent RNA. Radioactive tracer experiments (discussed in Chapter 11) have shown that the nucleolus is a site of active RNA synthesis, and that the RNA produced there is later found in the cytoplasm. There have been many speculations about the role of nucleolar RNA as intermediary between chromosomes and cytoplasm, but there are few data.

Kinetosomes: One class of particulate components of the cytoplasm which have been observed to divide, and not to arise *de novo*, are the basal bodies from which cilia and flagella develop, including the blepharoplasts (also called centrosomes), kinetosomes, and the kinetoplasts. Lwoff proposed that these are replicating units, on the basis of his meticulous morphological studies of the complex cycle of differentiation which these bodies undergo in ciliated protozoa. Instances have been reported of irreversible loss of basal bodies following treatment of the cells with the acridine dye, trypanflavin, and by dilution.

Recently, a cytochemical analysis of the kinetoplast in particularly favorable material—the parasitic flagellate, *Trypanosoma cruzi*—has demonstrated the presence both of DNA and of its typical chromosomal companion, a histone-type protein. The complete demonstration that these bodies are replicating organelles has not yet been made, but the chemical and morphological evidence suggests that eventually they will be established as cytoplasmic organelles which contain genetic information.

Mitochondria are membranous organelles structurally independent of other cytoplasmic components. They contain the cytochrome enzymes of terminal electron transport as well as enzymes of the citric acid cycle, of fatty acid oxidation, and oxidative phosphorylation; they are the principal energy source of the cell.

As shown in Plate XIV, each mitochondrion consists of two sets of

membranes, a smooth continuous outer coat, and a convoluted inner membrane, the folds of which consist of platelike double membranes called *cristae*. The number of *cristae* per unit length varies with the physiological state of the cell and probably is an adaptive, not a hereditary, property. The outer membrane is probably concerned with permeability control; the inner membrane, with the *cristae*, contains most if not all of the insoluble enzymes of respiration.

When mitochondria are treated with a suitable solvent, the structure is disrupted and small empty vesicles of assorted sizes are obtained. They contain the respiratory enzymes of the electron transport chain, and have been called electron transport particles, ETP. Most other enzyme activities of the mitochondria are lost in the deoxycholate treatment.

The ETP contains an array of insoluble enzymes which catalyze the oxidation of carbohydrate derivatives to produce energy utilized to drive other metabolic systems. The over-all oxidation of carbohydrate to CO_2 and H_2O is accomplished in the cell by a large number of successive reactions such that energy is released in small discrete packets at many steps. The terminal stages of this process, involving the transport of electrons from a carbohydrate donor to molecular oxygen, is accomplished by a chain of enzymes, like a bucket brigade. The enzyme components of this chain include at least two different flavoproteins and four different cytochromes. The function of electron transport suggests the need for precise spatial orientation of these enzymes, and recent evidence based upon studies of fragmented ETP supports this view.

Mitochondria have been observed to divide by transverse splitting, and it is a reasonable inference that they arise solely from pre-existing mitochondria; but there is no definitive evidence on this point. The presence in the membranes of many insoluble enzymes requiring a high degree of orientation certainly suggests that the enzymes are formed *in situ*. Evidence has been obtained for the formation of cytochrome *c* in isolated mitochondria (Chapter 11). One may speculate whether mitochondrial membranes are replicated in the sense of copying of the array of macromolecules on a supertemplate, e.g., a pre-existing bit of mitochondrial membrane. Since mitochondria vary considerably in size and *cristae* content in cells of the same genotype, depending upon physiological conditions, it is unlikely that the mitochondrion as a whole is the relevant unit in replication.

If mitochondria do replicate, are nucleic acids involved somewhere in the process? No nucleic acids have been detected in mitochondria by cytological or cytochemical techniques, but chemical analysis of carefully isolated mitochondria indicates that there is probably a small amount of RNA, approximately 1 per cent of the protein content. It is

not easy to distinguish such a low nucleic acid content from contamination by ribosomes, so one can only conclude that, if any RNA is present in mitochondria, there surely is not much. Further investigations will require the use of special techniques designed to follow the behavior of small numbers of molecules.

Chloroplasts, the organelles in which photosynthesis occurs in plant cells, have much in common with mitochondria: (1) they are both membranous structures existing free in the cytoplasm; (2) their membranes contain many insoluble components requiring precise spatial orientation for their functioning; (3) they contain small amounts of nucleic acid, if any; and (4) their mode of origin is not established. Many lines of evidence suggest that chloroplasts like mitochondria arise from pre-existing units of the same kind, not by *de novo* origin from undifferentiated cytoplasm.

Chloroplasts are surrounded by an envelope consisting of two smooth continuous membranes separated by a distinct space. The internal organization appears complex at first glance, and different from species to species. On careful examination, however, a basic plan common to all chloroplasts can be discerned. The structural unit is the *disc*, which can be visualized as a much flattened vesicle or as a pair of plate-shaped membranes joined at their ends to form a closed structure. In some algae, the chloroplasts consist of single stacks of 10 or 12 of these discs. In higher plants, chloroplasts contain many stacks of discs, each stack called a *granum*, oriented more or less precisely by thin membranes which run between the grana.

The discs are embedded in a matrix which contains the soluble enzymes involved in the conversion of photosynthetically reduced CO_2 to sugars and starch. The discs themselves enclose an inner space of unknown function and content, but presumably essential for photosynthetic activity. The macromolecular organization of the disc membranes has been a favorite subject of speculation for many years, but as yet we know only that it must be a multicomponent system, containing many insoluble enzymes of photosynthesis as well as chlorophylls, carotenoids, and phospholipids. It is a reasonable guess from structural considerations that the molecular composition of the disc membrane is asymmetrical in cross section, with chlorophyll present in a single layer on one side of the membrane. This idea fits well with some current hypotheses of the nature of the photochemical steps in photosynthesis.

Although the disc is probably a functional unit, it definitely is not a hereditary unit, since dark-grown strains of algae which contain no chlorophyll also contain no disc membranes. Nonetheless, such strains retain their ability to form normal chloroplasts when provided with light

and, consequently, with chlorophyll. During vegetative growth and multiplication, chloroplasts can be seen to increase in size and to divide. In plants, chloroplast fission has been described in young leaf cells, but chloroplasts are not transmitted as such through the zygote. In young seedlings, plastids develop from structures about the size of mitochondria, called preplastids. The origin of preplastids is unknown, so that at the morphological level it is not clear whether these organelles have a genetic continuity. If so, one would expect the preplastid to be a replicating unit and, presumably, to contain a nucleic acid. There is some cytochemical evidence suggesting the presence of RNA in chloroplasts, based upon staining reactions, but not confirmed by any other method.

Despite the lack of critical evidence, there is a body of indirect evidence implicating chloroplasts and, to a similar degree, mitochondria, in their own heredity. This evidence comes from the genetic literature, where some of the best examples of nonchromosomal heredity involve these organelles.

CHLOROPLAST HEREDITY

Since the chloroplast is such a complex organelle containing many pigments, enzymes, and perhaps enzyme-forming sites as well, it should be anticipated that normal chloroplast development may be interfered with in a large number of ways. For example, many chromosomal mutations are known which alter chloroplasts. In maize alone over 50 different loci of this sort have been mapped. In addition to Mendelian factors, instances of nonchromosomal inheritance of chloroplast mutations have been reported in a large number of plants.

Although many kinds of chloroplast abnormalities have been observed, those which are easiest to study genetically are the ones in which the plants are sectored, containing normal green as well as mutated stripes. The green areas provide enough products of photosynthesis to keep the plants alive until maturity and seed production occur. Both chromosomal and nonchromosomal factors induce striping.

The common type of nonchromosomally induced striping is called *status albomaculatus*; the results of reciprocal crosses between green and striped plants are as shown in Figure 9.4. The striped phenotype is not transmitted by the male parent, and, when the female parent is striped, three classes of progeny, green, white, and striped, are produced with variable frequencies. The same pattern of inheritance recurs gen-

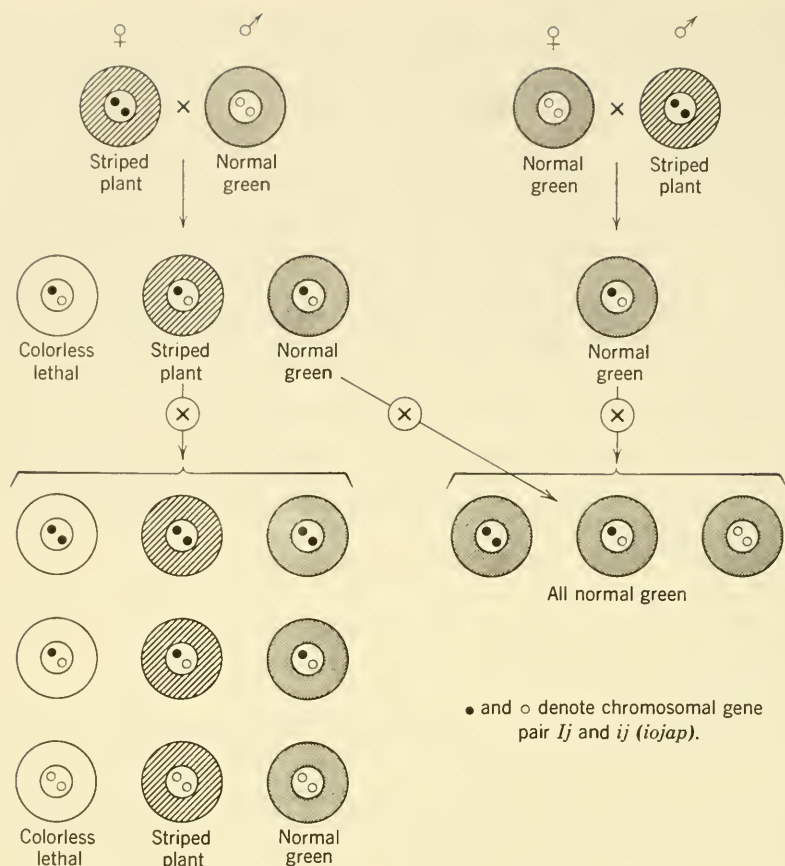


FIGURE 9.4. Nonchromosomal inheritance of striping in higher plants, so-called *status albomaculatus*. In reciprocal crosses between normal green and striped plants, when the genotype of the progeny is determined by the female parent, the genetic factor responsible for striping is nonchromosomal. When the female parent is normal green, the striping phenotype disappears; all progeny are green, and chromosomal genes segregate normally in the F₂ generation. When the female parent is striped, three classes of progeny appear in the F₁: colorless, striped, and green with variable frequencies. When the striped F₁ plants are self-pollinated, or crossed to any male, again three classes of progeny are found in variable proportions with chromosomal genes segregating normally (after Rhoades, 1946, *C. S. H. Symp. Quant. Biol.*, 11:202).

eration after generation, and clearly cannot be accounted for on a chromosomal basis.

Further evidence of the nonchromosomal determination of striping comes from a study of somatic segregation. In maize, for example, the

ear consists of rows of fertilized eggs, arising by mitotic divisions and spatially oriented, so that cell lineage relations are more or less clearly maintained. Each fertilized egg develops into a separate kernel. If an ear developing in a striped region is pollinated by a normal green male and the resulting kernels are planted in the form of a map, the germinating seedlings are again of three types, but spatially oriented by sectors, as shown in Figure 9.5. Thus hereditary determinants of green and of bleached chloroplasts are segregated among the egg cells, and this segregation is reflected in the distribution of green, white, and striped progeny seedlings in sectors.

In this system, both maternal inheritance and somatic segregation indicate the presence of a nonchromosomal determinant which influences chloroplast development. However, it does not indicate whether or not the determinant is actually located in the chloroplast; it may be anywhere in the cell except on a chromosome.

If the appearance of green and white stripes were the result of sorting out of normal and mutant plastids, one might expect to find cells with mixtures of plastid types at the borderline between sectors. Considerable effort by a number of investigators to study plastid segregation has not provided definitive results. In general, cells with mixed plastid types are not found, but rather there is an increasing abnormality of appearance of all plastids in cells close to the white sectors. These observations are more in line with the hypothesis of a cytoplasmic inhibitor of plastid formation than of plastid segregation.

A detailed study of the *iojap* mutants of maize by Rhoades was designed to investigate this question further. *iojap* is a chromosomal gene, which gives rise to striped plants when homozygous recessive. In crosses of the resulting striped plants reciprocally with green ones, the progeny seedlings show maternal inheritance of striping, as in the examples illustrated in Figure 9.4. In many generations of backcrossing striped females with green males, there is no observed effect of the male parent upon the progeny, despite the absence of the *iojap* gene after outcrossing. Planting ears in the form of a map, from sectorized plants pollinated by normal green males, gives evidence of somatic segregation as in Figure 9.5. Thus, in this system, the gene *iojap* produces a mutation, that is, an irreversible change, in a nonchromosomal determinant.

Indirect evidence that this determinant is not in the chloroplast itself comes from the following observations:

1. Cytologically one does not see cells with mixed green and mutant plastids at the boundaries of striped areas.
2. The extent of striping can be varied greatly by changing the grow-

gr	gr	gr	gr	gr		gr	gr		gr
gr		gr	gr		gr	gr			
gr	gr	gr	gr	gr	gr		gr	gr	
gr		gr	gr	gr		gr	gr	gr	gr
gr	gr	gr	gr			gr	gr	gr	gr
gr	gr	pa	pa	gr	gr	gr	gr	gr	gr
gr	gr	st	st		gr	gr	gr		gr
gr	gr	st	pa	st	gr	gr	gr	gr	gr
gr	gr	pa	pa	st	gr	gr	gr		gr
gr	gr	st	pa		gr	gr	gr		gr
gr	gr	gr	pa	gr	gr	gr	gr	gr	gr
	gr	pa	pa	pa	gr	gr	gr	gr	gr
	gr	gr	st	pa	gr	gr	gr	gr	gr
pa	gr		pa	st	gr	gr	gr	gr	gr
gr	gr	gr	pa	gr	pa	gr	gr	gr	gr
st	gr	gr	gr	st	pa	pa	gr	gr	gr
pa		gr	pa	st	pa	pa	gr	gr	gr
gr	gr	gr	st	pa	pa	pa	gr	gr	gr
gr	gr		pa	pa	pa	pa	gr	gr	gr
gr	gr	st	pa	st		pa	gr	gr	gr
	gr	pa	st	pa	pa	pa	gr	gr	gr
gr	gr	gr	st		pa	pa	gr	gr	gr
gr	gr	pa			pa	gr	gr	gr	gr
gr	pa	gr	gr			st	gr	gr	gr
gr	gr	st	pa			st	st	gr	gr
gr	pa	gr				pa	gr	gr	gr
gr	gr	st	gr			pa	pa	gr	gr
gr	pa	pa	gr			gr	st	gr	gr
gr	gr	st					pa	gr	gr
gr	gr		gr			st	st	gr	gr
gr	gr	gr	gr			st	st	gr	gr
gr	gr		gr			gr	gr	gr	gr
st	st	gr	gr				gr	pa	pa
pa	st	gr	gr			st	st	st	st
st	gr	st				pa	st	pa	pa
gr	gr	gr				gr	gr	gr	gr
st	gr	gr				st	st		
st	gr	gr					pa	st	st
st	gr	pa					st	pa	pa

FIGURE 9.5. Maize kernels from a self-pollinated ear of a striped plant, planted as a map of the 10-rowed ear. Seedling classes (*gr* = green, *st* = striped, *pa* = pale green) show sectoring in their distribution. Possible position of sectors indicated by dotted lines. This is evidence of somatic segregation of a genetic determinant. Blank spaces are missing kernels (after Anderson, 1923, *Botan. Gaz.*, 76:411-418). (Copyright 1923, by the University of Chicago.)

ing conditions. For example, summer field-grown plants are much greener, with fewer white sectors, than sibling plants grown in the winter in the greenhouse. Random segregation of pre-existing normal and mutant plastids cannot readily explain such an environmental influence on the extent of white sectoring.

3. When the proportion of white seedlings is compared in the first and subsequent generations of crossing striped plants, it is found that the frequency of white increases with the numbers of generations of female striped plants, as if some toxic substance were increasing slowly in concentration, affecting more and more of the determinants. Moreover, the per cent of entire white seedlings in each generation is much less than the per cent of white areas in the striped seedlings, again indicating a process more complex than mere random segregation of particles.

These observations favor the view that the nonchromosomal determinant in this system is not the plastid itself.

Currently, the problem of chloroplast heredity is being re-examined with chloroplast mutants of microorganisms. The finding that streptomycin-treated seeds give rise to albino and striped seedlings led some investigators to test the effects of streptomycin upon algae. Strains of *Euglena* were found which responded dramatically to streptomycin treatment by the irreversible loss of chlorophyll-forming ability. Subsequently, the same result was obtained in these strains by a short heat treatment at 34°C and also by UV-irradiation. In all three treatments, the effects were exhibited by most of the treated cells, with very little death. Thus, it seems that an induced change, different from spontaneous mutation in affecting an entire treated population, has resulted in loss of a hereditary property. It is not established whether the chloroplast itself or some other cell constituent is the site of the altered or lost genetic determinant; the eventual loss of ability to form chloroplasts may be a secondary effect of a primary change elsewhere. In the absence of a means to study recombination in this asexual organism, no method has been devised to locate the relevant hereditary site.

In *Chlamydomonas*, streptomycin treatment has given rise to yellow mutants, which have lost their ability to carry out a particular step in chlorophyll synthesis. These mutants are phenotypically the same as previously studied yellow mutants of spontaneous origin. In crosses between spontaneous and streptomycin-induced yellow mutants, no green recombinants have been found, indicating that the same site has been altered in both groups of mutants. In crosses of yellow mutants with normal green strains, the progeny segregate 1:1, but the *y* factor shows only first-division segregation. Since there is but one chloroplast per

cell, it is possible that the regular segregation observed is that of an oriented nonchromosomal factor. This possibility is raised for two reasons. First, the induced character of the mutation suggests that it is nonchromosomal, since the weight of evidence supports the view that chromosomal gene mutability is a random process. Second, the exclusive occurrence of first-division segregation locates the y_1 factor as either very close to a chromosomal centromere or else as nonchromosomal but still replicating and segregating in a regular way. Only the occurrence of linkage with some chromosomal gene can resolve this question, and in tests thus far of seven chromosomes (out of eight to ten) no linkage has been found.

In summary, the nonchromosomal determination of some chloroplast properties has been well documented in many species of plants, but in no system has the identification of the determinant been established. Because of the organized complexity of chloroplast structure, it seems likely that some aspects of chloroplast heredity may be determined by copying on a supertemplate of some sort. If such a mechanism exists, it should be localized within the chloroplast. Nonchromosomal genes or steady-state systems influencing chloroplast development, on the contrary, might be found elsewhere in the cell.

MITOCHONDRIAL HEREDITY

The basic issues of mitochondrial heredity are very similar to those we have just been considering with respect to chloroplasts: (1) Where are the determinants located which control mitochondrial formation, and (2) do mitochondria replicate? Here too, as with chloroplasts, there is good genetic evidence that both chromosomal and nonchromosomal determinants contribute to mitochondrial development, but neither the location nor chemical nature of the nonchromosomal determinants has been established.

The best genetic evidence about mitochondrial heredity comes from studies of the poky mutants in *Neurospora* and the petite mutants in yeast, which were described above. The systems have certain features in common, among them the occurrence of altered cytochrome enzymes in the mutant strains and the existence of chromosomal as well as non-chromosomal genes with very similar phenotypic effects. A comparison of the cytochrome components of a number of these strains is given in Figure 9.6.

In addition to the nonsegregation in crosses, a very important evidence

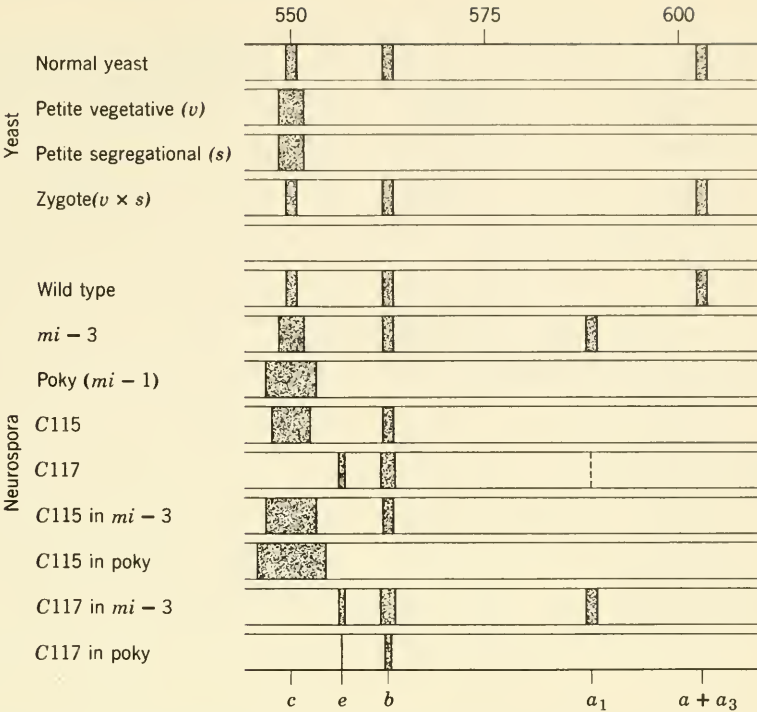


FIGURE 9.6. Cytochromes of normal and mutant strains of *Neurospora* and yeast. The absorption bands of the cytochromes are indicated, the wider bands representing higher concentrations. The actual differences are larger than indicated. The positions of the bands of some known cytochromes, c, e, b, a₁, and a + a₃, are shown at the bottom of the figure (from Wagner and Mitchell, 1955, *Genetics and Metabolism*, New York, Wiley, p. 334).

of the nonchromosomal nature of the petite factor comes from studies of its origin. Ephrussi has shown that the petite mutation can be induced by treating normal yeast cells with eufavin, the most direct demonstration being by means of pedigrees of the sort shown in Figure 9.7. The mode of action of this acridine dye is not known, but its ability to convert a population of normal yeast cells to petites at a concentration which does not kill is a dramatic example of induced mutation, quite distinct from the mutagen-induced chromosomal gene mutations which occur at a frequency of 10⁻⁶ or less.

The change induced by eufavin does *not* involve the loss of mitochondria as morphological entities. Both in electron micrographs and in

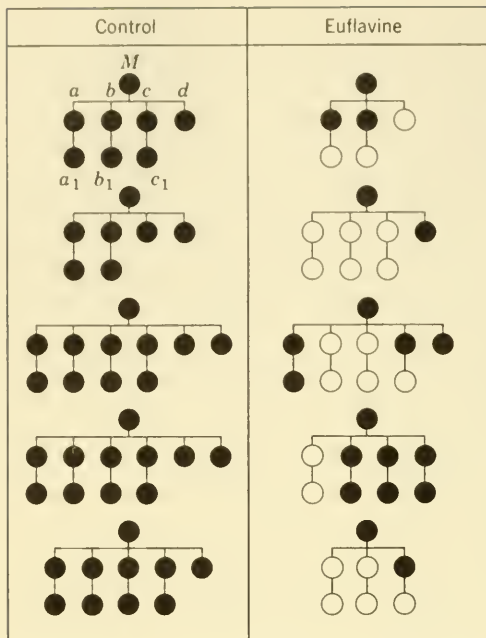


FIGURE 9.7. Induction of petite mutations by eufлавин. Pedigree analysis of single yeast cells and their descendants grown in the presence and absence of eufлавин. The first line of descendants represents the buds produced by each treated cell, and the second line represents one bud from each of the first generation of buds. The phenotype of each cell was determined, by tests of the clone developing from it, as the presence or absence of cytochrome oxidase. Black circles represent wild type clones; open circles represent petite mutants (from Ephrussi and Hottinguer, 1950, *Nature*, 166:956).

cell extracts, particles with the morphological properties of mitochondria are found in petite cells, but they are inactive in terminal oxidation. The cytochrome spectrum of petite cells resembles that of normal yeast grown anaerobically, and, consequently, one might expect that studies of the shift from anaerobic to aerobic conditions in normal yeast could shed some light on the nature of the petite mutation. Recently Slonimski has reported evidence of a *conversion* of one of the enzymes in this pathway—the flavoprotein, lactic dehydrogenase—from its anaerobic form to its aerobic form when oxygen is supplied, rather than *de novo* synthesis of the aerobic enzyme.

Petite mutants resulting from a chromosomal gene mutation have been called “segregational petites,” in distinction to the “vegetative petites” which result from alteration in a nonchromosomal gene. In crosses of segregational $\times$ vegetative petites, the diploid zygote is normal in phenotype, and the progeny arising after meiosis segregate 2:2 for the chromosomal gene. These results are precisely the same as those observed in crosses of segregational petites $\times$ normal strains. An important inference from these results concerns the maintenance of the nonchromosomal de-

terminant in the segregational petite strains. In order to obtain a normal diploid from the fusion of a segregational with a vegetative petite, the segregational petite must contribute the nonchromosomal factor which the vegetative petite lacks. Thus, in this system, it is shown that nonchromosomal genes are maintained and replicated despite their inactive status, just as are the chromosomal genes.

In the mutants of *Neurospora* with altered cytochrome activities, the morphological picture is quite different. Here it seems likely that some structural alteration in mitochondrial organization is involved in the phenotype. No mitochondria comparable to those of wild-type strains are found in the poky mycelia, although cytochrome activity is associated with sedimentable membranes which are present throughout the cytoplasm. Thus, the altered cytochrome activity may be a secondary consequence of some more basic defect.

A related finding of interest comes from the mycelial fusion of wild-type strains with poky, forming what have been called heterocytosomes because they contain two kinds of cytoplasm. Initially these fused mycelia grow at the wild-type rate, but gradually they slow down, and eventually become typically poky mycelia. In subsequent crosses, only the poky factor is transmitted. Thus, the mutant elements replace the wild type, and the nuclear composition has no effect upon the outcome. This result is surprising since one would expect to find selection operating against the mutant. In heterocytosomes composed of two mutant strains, poky and *mi-3* (a different cytochrome-deficient nonchromosomal mutant), the growth rate is slow, indicating that although these mutations differ in some way, as shown by their cytochrome activity patterns, they are not complementary. Another pair of nonchromosomal mutants, *mi-3* and *mi-4*, do complement in the heterocytosome.

Taken together, these studies indicate that mitochondrial heredity can be influenced by mutation of either chromosomal or nonchromosomal genes. However, as with chloroplasts, there is as yet no definitive information on the location or identity of the responsible nonchromosomal determinants.

NONCHROMOSOMAL HEREDITY INVOLVING NO KNOWN STRUCTURES

In the early investigations of nonchromosomal heredity in higher plants, a large number of morphological phenotypes were involved, especially in interspecies crosses which provided a wide diversity of traits

exhibiting maternal inheritance. The most widespread of these traits is pollen sterility, which has been described in some 20 to 30 different genera, beginning with Correns' first example reported in 1909 in *Cirsium*. To illustrate the principles involved, as well as some of the complications, let us consider the most extensively investigated systems, those in the corn plant, *Zea mays*.

Cytoplasmically inherited male sterility in maize was first reported by Rhoades in 1933, on the basis of maternal transmission. Plants of the male-sterile line, when outcrossed with normal pollen, gave almost entirely male-sterile plants. Repeated backcrosses with fertile pollen did not restore fertility. Occasionally some fertile pollen was produced in such crosses, and this provided an opportunity to test transmission of male sterility through the pollen. As predicted from the absence of segregation among the progeny of male-sterile female parents, the trait was found to be transmitted only from the mother and not through the exceptional fertile pollen grains formed on the male-sterile plants.

More recently, a number of new male-sterile strains of independent origin exhibiting nonchromosomal inheritance have been studied. Most of these appeared as sterile tassels on single plants in large commercial fields of inbred corn, strongly suggesting an origin by spontaneous mutation. The effect of the mutation appears only after meiosis, interfering with maturation of the pollen grain.

Somatic segregation of the pollen-sterility determinant can be seen simply by the appearance of fertile and sterile sectors on the tassel, and represents evidence of the particulate nature of the determinant. A most interesting example of sectorized pollen sterility is that appearing in plants carrying the *iojap* gene, which also induces plastid mutations. In recessive *iojap* plants, both cytoplasmic pollen sterility and plastid mutations occur in striped sectors, but the sectors do not coincide, indicating that different particulate segregating elements, both susceptible to mutation by products of the *iojap* gene, are involved in plastid heredity and in pollen development.

In recent studies of cytoplasmic male-sterility in maize, some chromosomal genes have been found which are fertility restorers (*fr*). There is specificity of response, because particular genes can restore fertility only with particular male-sterile lines. In all cases, the gene-restorer action is solely phenotypic; it does not interfere with the replication or maintenance of the nonchromosomal determinant. As shown in Figure 9.8, a male-sterile strain crossed with a fertility restorer gave all male-fertile plants, but when the *fr* gene was subsequently crossed out of the stock, the male sterility reappeared. Both nonchromosomal male-sterility and the chromosomal-restorer genes are of great commercial

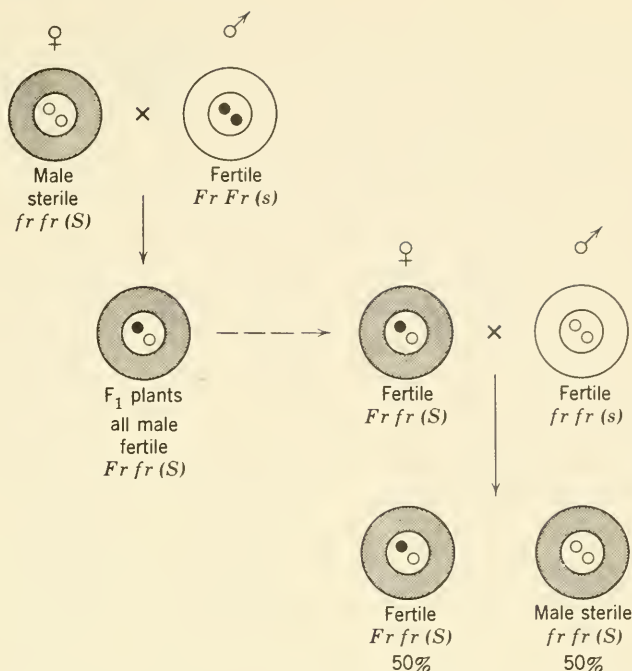


FIGURE 9.8. Inheritance of nonchromosomal male sterility in maize. Female plants carrying the male sterility factor (S) and recessive chromosomal genes fr (fertility restorer) crossed with fertile males carrying the dominant gene Fr give rise to F_1 progeny which are male fertile. When such F_1 's are used as the female parent in crosses with fertile males which are fr , the progeny in the next generation segregate one fertile:one male sterile, indicating that the sterility factor was transmitted unexpressed through the male fertile F_1 plants (after Jones, 1950, *Genetics*, **35**:507).

value in hybrid seed production, eliminating the most costly hand-labor step, namely, the detasseling of the ear-bearing female parent.

In summary then, nonchromosomal male sterility represents a hereditary system which shows maternal inheritance and somatic segregation of some unidentified determinant which is capable of mutation to more than one alternative state.

The nonchromosomal determination of streptomycin resistance in *Chlamydomonas* is another system not associated with any known structure. Although streptomycin affects chloroplast inheritance, as discussed above, no evidence has been obtained that the site of streptomycin resistance is related to the chloroplast. No apparent morphological differences between streptomycin-sensitive and streptomycin-resistant strains have been detected in comparative electron microscope studies.

The close phenotypic interaction of chromosomal and nonchromosomal genes is illustrated in this system by a chromosomal gene, *A*, which increases the streptomycin-resistance level of *sr-500* strains and of chromosomal *sr-100* strains as well.

Other examples of nonchromosomal genes have been investigated in the molds, for example, genes which determine senescence and barrage in *Podospora*. Senescence, the death of aging mycelia, is a common occurrence in many molds. In *Podospora*, it is possible to make reciprocal crosses between the fruiting bodies of aging and of young mycelia, and thereby to study the inheritance of senescence. It is found to be maternal. Further evidence of its nonchromosomal nature comes from mycelial fusions between young and senescent hyphae. In the absence of nuclear migration, there is rapid infection of the senescent mycelium by hereditary determinants which convert it back to the youthful state, as shown by subsequent crosses. Thus, in these strains, senescence is the result of the loss or alteration of a nonchromosomal hereditary determinant, capable of propagation by infection through a mycelial fusion.

In the barrage phenomenon (Figure 9.9), strains of *Podospora* having mycelia which form a line at the point of contact are found to differ by a single gene pair, *S/s*. However, in crosses of strain $S \times s$, the *s* phenotype is lost and in its place a new class of progeny appear in which the mycelium is compatible both with *S* and with *s*. This new phenotype, called *s^s*, must be attributed to a genetic alteration since it is stable in vegetative growth and in crosses. However, it shows strictly maternal inheritance.

Although the *s^s* property is stable, occasional mutations occur, always back to *s*. Most interesting, the *s^s* property can be lost by infection. When mycelial grafts are made between *s^s* and *s* strains, the *s* property rapidly spreads through the *s^s* mycelium, in the absence of nuclear migration, converting the *s^s* strain completely back to *s*. It is inferred that the *s^s* property results from the loss of a cytoplasmic determinant from the *s* strain, owing to interactions with *S* cytoplasm at meiosis and, as a result, the *s* mycelium becomes compatible with *S*. Evidently, this determinant can rapidly re-establish itself in an *s^s* mycelium after re-introduction by fusion.

Barrage is of particular interest because it combines the features of an apparent gene mutation occurring at meiosis with maternal inheritance of the mutation, and infectivity of a cytoplasmic factor. The apparent gene mutation turns out upon analysis not to be chromosomal at all, but rather it is a cytoplasmic change resulting from gene interaction in the ascus at the time of meiosis. Formally, the apparent 100 per cent muta-

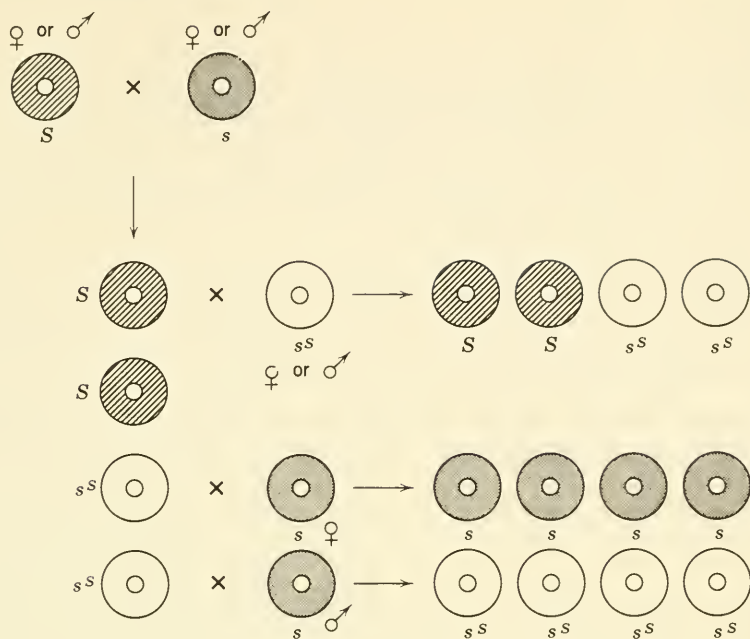


FIGURE 9.9. Origin and inheritance of "barrage" in *Podospora*. Mycelia of *Podospora* produce male and female structures as in the related fungus *Neurospora*, and consequently reciprocal crosses may be made between two strains of opposite mating type. In crosses of $S \times s$, the progeny segregate $2S:2s$, with the total loss of the s phenotype, and appearance in its place of the new property s^s . In reciprocal crosses of $s^s \times S$, the progeny segregate $2:2$ normally. However, in crosses of $s^s \times s$, the F_1 progeny all resemble the female parent, thereby showing nonchromosomal inheritance of the difference between s and s^s . Chromosomal genes segregate normally in these crosses (after Rizet, Marcou, and Schecroun, 1958, *Bull. Soc. Française Physiol. Végétale*, 4:136).

tion at meiosis, a highly unusual occurrence, resembles the paramutation phenomenon recently discovered in maize. No cytoplasmic component has been identified in paramutation, but one may surely wonder, nonetheless, whether the system has an explanation like that of barrage.

An interesting aspect of the *Podospora* work is the evident infectivity of the nonchromosomal determinants. The relationship between infectivity and heredity is receiving widespread attention at present, and has fundamental implications both in heredity and in disease. Let us turn then to a consideration of other infectious hereditary agents which are also extrachromosomal.

INFECTIOUS AGENTS

There are a number of striking examples in the literature of host-cell traits determined by infectious particles. In the past, it was considered not only possible but essential to distinguish between normal cell constituents and intracellular parasites of various sorts. One criterion generally employed was infectivity.

Suppose we define as an infectious agent any hereditary material capable of entering a cell from outside and replicating within it. An example of such an agent is DNA itself, as employed in transformation experiments; and the ultimate example is the sperm nucleus in fertilization. If instead of replication one takes cell damage as the criterion, the situation is no clearer: consider the lysogenic bacteria, in which the virus, when it is in the prophage state, contributes no damage whatsoever but often some positive properties such as special new antigens. Indeed, all recent work in this field has demonstrated the futility of attempting to distinguish between normal cell constituents and infectious agents. All one can do is to investigate each genetic system for what it is, hoping ultimately to construct a picture of the cell in which the various jig-saw pieces fall into place. In the following discussion, we shall briefly consider a few outstanding examples of infectious determinants.

Kappa

In 1943, Sonneborn reported cytoplasmic inheritance in *Paramecium aurelia* of a trait called *killer*, recognized as the ability of some strains to kill others. To understand the method of genetic analysis employed, it is necessary to consider first a simplified account of the principal features of mating and meiosis in *Paramecium*, as diagrammed in Figure 9.10.

Each organism contains two diploid micronuclei and one large macronucleus, a cell organelle unique to ciliated protozoa, containing many replicates of the diploid genome. Conjugations involve pairing of two animals at the anterior end and in the paroral cone (mouth) region. After pairing begins, the macronuclei of both mates disintegrate, and the micronuclei undergo meiosis, producing eight haploid nuclei per animal; any one of these nuclei may survive, the other seven degenerate. The remaining nucleus in each animal divides mitotically, and one of the two daughter nuclei passes across the paroral cone into the other mating cell. Each mate then contains two haploid nuclei, which fuse to produce a diploid fusion nucleus containing one chromosome comple-

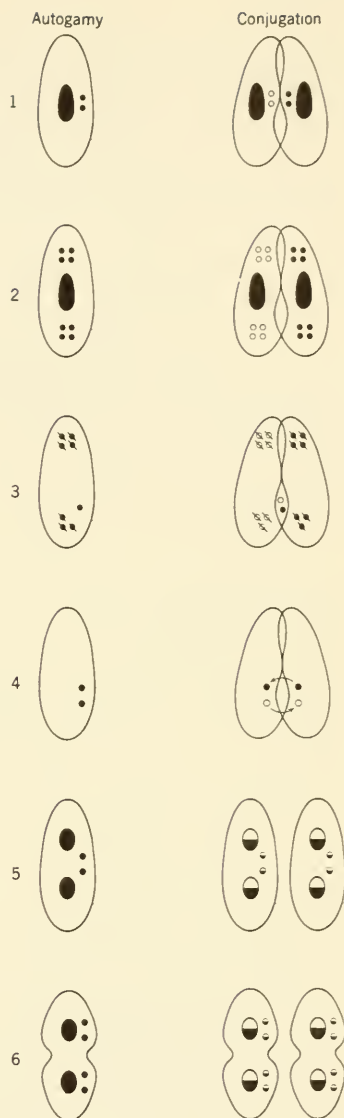


FIGURE 9.10. Autogamy and conjugation in *Paramecium*.

1. Animals with one macronucleus and two micronuclei per cell.

2. Each micronucleus undergoes meiosis. Result: eight haploid nuclei. The macronucleus begins to degenerate.

3. Seven of the nuclei degenerate, and the eighth will divide.

4. In conjugating animals, one member of each pair of remaining nuclei is exchanged.

5. The haploid nuclei fuse to form the new diploid micronucleus, which divides twice, giving rise to two new macronuclei and two micronuclei.

6. At the next cell division, one macronucleus goes to each daughter cell, but the micronuclei divide again so that two are present in each cell.

ment from each parent. Each fusion nucleus divides twice, producing four diploid nuclei, two of which remain as micronuclei, and two of which enlarge, becoming the new macronuclei. The micronuclei divide once more so that upon cell division each daughter has one macronucleus and two micronuclei.

Generally little or no cytoplasm is transferred at conjugation, but under special conditions cytoplasmic transfer can be induced by a prolonged persistence of the paroral bridge.

It is also possible to induce meiosis in unmated individuals, and this process, called autogamy, leads to the segregation of any heterozygous genetic factors present, with the consequent establishment of genetically homozygous animals. The details of the process are rather similar to those of conjugation, as can be seen in Figure 9.10. Genetic segregation in conjugation and in autogamy are diagrammed in Figure 9.11.

Inheritance of the killer trait is shown in Figure 9.12. When no cytoplasm is transferred during conjugation, the killer trait is transmitted to those progeny receiving their cytoplasm from the killer-type parent, whereas in the event of cytoplasmic exchange, all progeny become killers. Thus, the inheritance of the killer property strictly follows transmission of cytoplasm, and is independent of gene segregation, provided the genotype will support the cytoplasmic factor, *kappa*.

Further intensive studies of *kappa* have supported the inferences of the first crossing experiments, namely, that *kappa* is a cytoplasmic determinant responsible for the killer trait. It is a large body some $0.2\ \mu$ in diameter, containing DNA and protein, and it can be isolated from killer cells and introduced into sensitive cells, converting them to killers; thus *kappa* is an infectious agent. Furthermore, *kappa* can divide at a different rate from that of the organisms themselves, giving rise to animals with increased numbers of particles and, what is more interesting, to animals with no particles at all.

Two single gene pairs have been identified which influence *kappa*. The better known of these, *K*, is necessary for maintenance of *kappa*, and cells containing only *k* lose their *kappa* particles and become sensitive, as shown in Figure 9.12.

Kappa may mutate to one of several forms which differ in the manner of killing sensitive animals, and segregation has also been demonstrated of two different mutant forms of *kappa* out of one animal.

A great deal has been written about the significance of *kappa*. Initially it was introduced as a particularly clear example of cytoplasmic inheritance, but when its size and chemical composition were discovered, it was relegated to the more specialized position of intracellular symbiont. More recently, as new particles are found in many organisms which are both infectious agents and the source of new and useful host phenotypes, the distinction has lost its meaning. *Kappa* is an extreme example of a class of genetic determinants which are not essential cell constituents, but which produce new traits in the host organism when they are present. Other examples include CO_2 sensitivity and altered

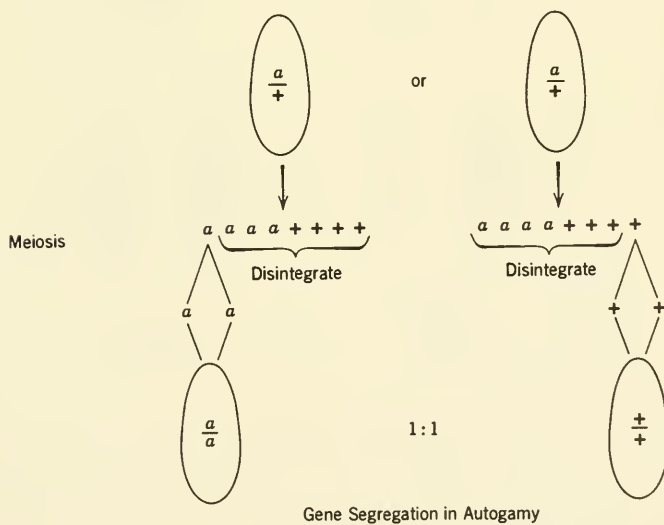
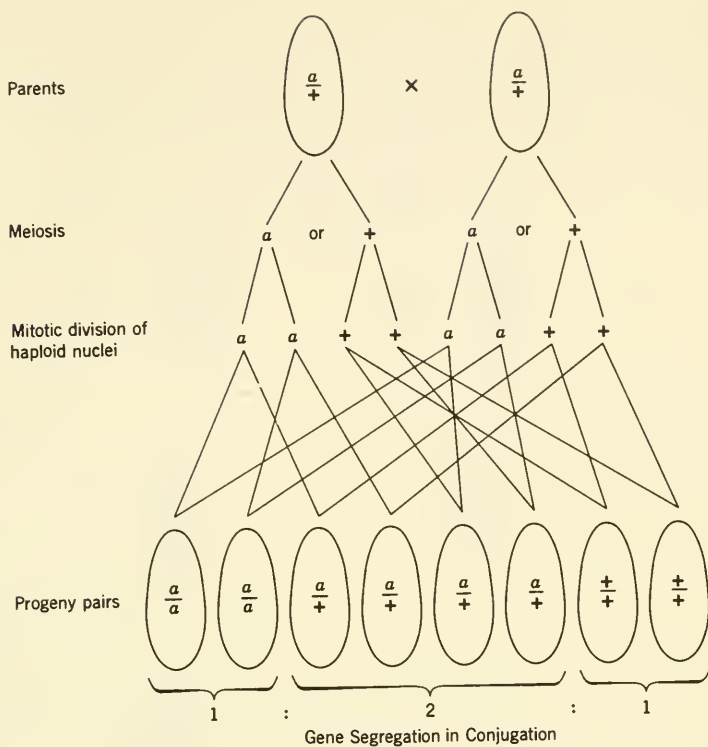


FIGURE 9.11. Gene segregation at autogamy and conjugation in *Paramecium*.

sex ratios in *Drosophila*, sex determination in bacteria, and toxin production by the diphtheria bacterium.

Both the *F* factor and lysogenic phage, discussed in Chapter 5, are examples of infectious agents which can exhibit a high degree of inte-

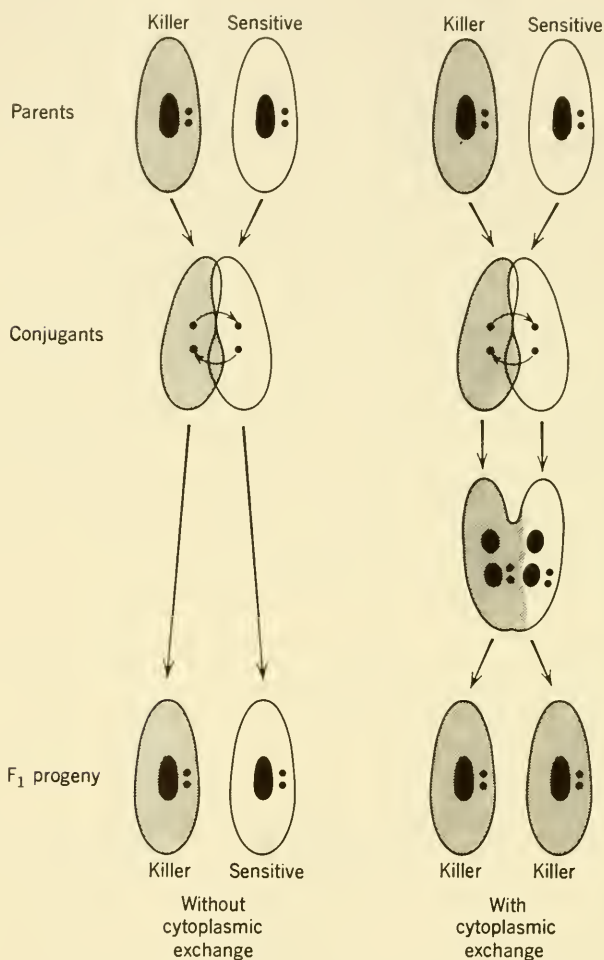


FIGURE 9.12. Cytoplasmic inheritance of the killer trait. Killer cells are shaded, sensitive cells clear. In the usual conjugation, there is exchange of nuclei but little or no cytoplasmic exchange. The phenotype of the F_1 progeny with respect to the killer trait is determined by the parent which contributed the cytoplasm. However, if extensive cytoplasmic exchange is allowed to occur, then all the F_1 progeny inherit the killer trait (after Beale, 1954, *The Genetics of Paramecium Aurelia*, Cambridge, The University Press).

gration with their host by attaching in a mapable position to the bacterial chromosome. They can also exist in an infectious and unintegrated form not attached to the bacterial chromosome. When the phage is unintegrated, it replicates rapidly free of cellular regulation and eventually kills the cell. The replication rate of *F* is not known, but indirect evidence suggests that it can multiply out of synchrony with the host cell. The term *episome* was coined initially to generalize from the behavior of these two kinds of infectious particles.

Sex Ratio in *Drosophila*

An example of a trait with considerable evolutionary implications, determined by an infectious particle, is "sex ratio" in *Drosophila*, in which female flies give rise largely or exclusively to female progeny. The trait is transmitted only by the female. Any exceptional male progeny of sex-ratio females do not transmit the trait to their offspring.

Examination of fertilized eggs laid by sex-ratio females reveals three classes: normal developing fertilized eggs, and abnormal dying fertilized eggs in about a 1:1 ratio, and in addition, considerable numbers of unfertilized eggs. The abnormal dying fertilized eggs are the best source of the sex-ratio agent, which can be transmitted by injecting cytoplasm from these eggs into normal females. Injected females begin to give broods consisting entirely or largely of females after a latent period of 10–14 days. The female sex-ratio progeny from injected females do not differ from other sex-ratio females, transmitting the factor to their offspring with the same high efficiency.

The abnormal fertilized eggs probably represent the male zygotes, that is the XY zygotes, and their susceptibility to the sex-ratio agent can explain the absence of males among the progeny of sex-ratio females. This hypothesis is strongly supported by the fact that cytoplasm from dying eggs, which converts normal females to sex-ratio, is capable of killing even adult males.

There are strain differences in susceptibility to the sex-ratio agent: some strains are more favorable than others for establishment of the agent after infection, and some strains are so susceptible that XX tissues are damaged in the female germ line, so that unfertilized eggs are killed and females are consequently sterile. These differences in strain susceptibility probably reflect gene-determined influences on rate of replication of the infectious agent.

The sex-ratio agent is particularly interesting because of the evidence that different tissues of the fly respond differently to it. It can only be found in the female germ line in infected flies, perhaps because it

can multiply only there, or perhaps because it is integrated and therefore noninfectious in other tissues. The great difference in susceptibility between XX and XY tissues may also be interpreted in terms of different rates of replication or degrees of integration of the agent with the host.

The infectious sex-ratio agent has just recently been described, and future investigations may be expected to provide new evidence on these key questions of replication and integration of an infectious determinant with the hereditary material of its host. Chromosomal genes which influence sex ratio are also known and have been extensively investigated.

CONCLUDING REMARKS

In this chapter we have considered two problems: the demonstration that nonchromosomal determinants exist, and the further question of their properties. The principal criterion we have chosen for identifying the class of nonchromosomal determinants is the inability to locate the factor on a chromosome. Applying this criterion has netted us a heterogeneous collection of systems which require further analysis. Nonmapability is a safely inclusive criterion for initial selection because any factors which do map are chromosomal. Further supporting evidence adduced has varied from system to system, and includes: (1) persistence and stability of the phenotype under conditions of cell reorganization (fertilization and meiosis) and environmental change; (2) mutability, especially a very high rate of mutation induced by a particular chemical; (3) infection in the sense of cytoplasmic spread; (4) uniparental transmission; and (5) somatic segregation not attributable to chromosomal events.

Approximately 100 well-characterized instances have been reported of determinants which meet one or more of these criteria, but from the existing information, it is fairly evident that they include different sorts of systems. In an attempt to organize this material into categories which might be useful for further analysis, we have considered four hypotheses proposed by different investigators at different times, concerning the nature of these systems. They are not mutually exclusive; it is even likely that examples of all four types may coexist within an organism simultaneously.

One property shared by several nonchromosomal determinants is their high rate of mutation or loss following treatment with particular chemical mutagens. The acridine dyes, such as euflavin, tryptaflavin, and acridine

orange, induce mutations to nonchromosomal petites, loss of the *F* factor in *E. coli*, and in one study, loss of the kinetoplast in a trypanosome. Streptomycin induces loss of chlorophyll-forming ability in many plants.

No critical evidence has been found as yet to support the super-template hypothesis, but considerations of the highly organized state of chloroplast and mitochondrial membranes and the low content (if any) of RNA, as well as the observed origin of these organelles by splitting from pre-existing ones, have led to the prediction that a novel mechanism of replication may be involved. In kinetosomes and other basal bodies, the presence of nucleoprotein indicates that these bodies may represent a sort of extranuclear chromosome.

Infectious particles such as *kappa*, which are not well integrated with the cell and can multiply at an asynchronous rate, represent one extreme of a group of viruslike particles which are infectious and nonmappable. In examples like the determinants of CO₂ sensitivity and sex ratio in *Drosophila*, neither their relation to normal cell constituents nor their indispensability is known. Lysogenic phages and *F* factors in *E. coli* have both chromosomal and nonchromosomal states, but no particles with this property have been identified in other systems. Because of their infectivity, this class of determinants can be most readily identified, but even here little information is yet available about the chemical constitution, mode of replication, and cell localization of most of the systems of this type.

Much more difficult methodological problems are involved in distinguishing between steady-state systems and those determined by replicating nonchromosomal genes. Catcheside has referred to the "conditional stability of the steady state," which we can contrast with the "unconditional stability of a gene." How can we sort them out experimentally? The physical or chemical characterization of the determinant is required, but has not been achieved in any of these systems as yet. In our view, such systems as those determining nonchromosomal male sterility, streptomycin resistance, and cytochrome abnormalities probably represent examples of "genes without chromosomes" rather than steady-state systems, because of their "unconditional stability" regardless of environment, state of expression, phenotypic interaction with other genes, or cellular reorganization following fertilization and meiosis.

Two systems have been described which may illustrate the inheritance of a steady state. One involves antigenicity in *Paramecium*, and the other, maintenance of a particular rate of enzyme formation in *E. coli*. Their properties differ sharply from those discussed in this chapter; they will be considered in Chapters 11 and 13.

BIBLIOGRAPHY

- Adaptation in Micro-organisms, 1953. *Third Symposium of the Society for General Microbiology*. Cambridge, The University Press, 339 pp. (Articles by Slonimski and Pollock.)
- Beale, G. H., 1954. *The Genetics of Paramecium aurelia*. Cambridge, The University Press, 178 pp.
- Correns, C., 1937. Nichtmendelnde Vererbung. In: *Handbuch Vererbungswissenschaft*. Vol. 2, pp. 1-159.
- Ephrussi, B., 1952. The interplay of heredity and environment in the synthesis of respiratory enzymes in yeast. *Harvey Lectures*, **46**:45-67.
- Ephrussi, B., 1953. *Nucleo-Cytoplasmic Relations in Micro-Organisms*. Oxford, Clarendon Press, 127 pp.
- Lwoff, A., 1950. *Problems of Morphogenesis in Ciliates*. New York, Wiley, 103 pp.
- Morgan, T. H., 1908-1909. What are "factors" in Mendelian explanations? *Amer. Genetic Assn. 5th Annual Report*.
- Porter, K. R., 1957. The submicroscopic morphology of protoplasm. *Harvey Lectures*, **51**:175-228.
- Rhoades, M. M., 1933. The cytoplasmic inheritance of male sterility in *Zea mays*. *J. Genet.*, **27**:71-93.
- Rhoades, M. M., 1946. Plastid mutations. *C. S. H. Symp. Quant. Biol.*, **11**:202-207.
- Rhoades, M. M., 1955. Interaction of genic and non-genic hereditary units and the physiology of non-genic inheritance. In: Ruhland, W. (ed.), *Handbuch der Pflanzenphysiologie*, Vol. 1. Berlin, Springer Verlag, pp. 19-57.
- Sager, R., 1958. The architecture of the chloroplast in relation to its photosynthetic activities. *Brookhaven Symp. Biology*, **11**:101-117.
- Sager, R., 1960. Genetic systems in *Chlamydomonas*. *Science*, **132**:1459-1465.
- Sonneborn, T. M., 1947. Recent advances in the genetics of *Paramecium* and *Euplotes*. *Adv. Genet.*, **1**:264-358.
- Sonneborn, T. M., 1951. The role of the genes in cytoplasmic inheritance. In: L. C. Dunn (ed.), *Genetics in the Twentieth Century*. New York, Macmillan.
- Wettstein, D., 1958. The formation of plastid structures. *Brookhaven Symp. Biology* **11**:138-159.

10 Some Mechanisms of Gene Action

How are the hereditary potentialities of a cell expressed? What are the chemical pathways leading from genotype to phenotype? In the preceding chapters, emphasis has been placed primarily upon analysis of the genetic material itself. Nonetheless, phenotypes have been intimately involved in all experiments, since the presence, absence, or mutated state of the genes could only be assessed in terms of their expression. Even experiments in which nucleic acids were treated *in vitro*, required a resulting phenotypic change to permit a genetic interpretation.

Thus, in fact, all genetic investigations have involved considerations of gene action, but investigators of transmission genetics have usually preferred to regard phenotypes merely as formal “markers” by means of which to follow the genes. This approach resulted in part from the dismaying complexity of the problem, for most mutations studied in higher organisms have involved intricate morphological changes such as altered bristle number or wing shape in *Drosophila*, or taillessness in mice.

THE SEARCH FOR ANALYZABLE TRAITS

The first problem in the analysis of gene action has been the choice of suitable phenotypic traits, amenable to study. With higher organisms, the most extensive studies have dealt with pigment changes, for example, alterations in the content of anthocyanins and anthoxanthins in plants following mutation at individual loci. It was shown in a series of researches, which began as early as 1913, that a single gene mutation could block the formation of a single pigment and, in some instances, of more than one pigment. In the primrose, for example, a magenta-colored

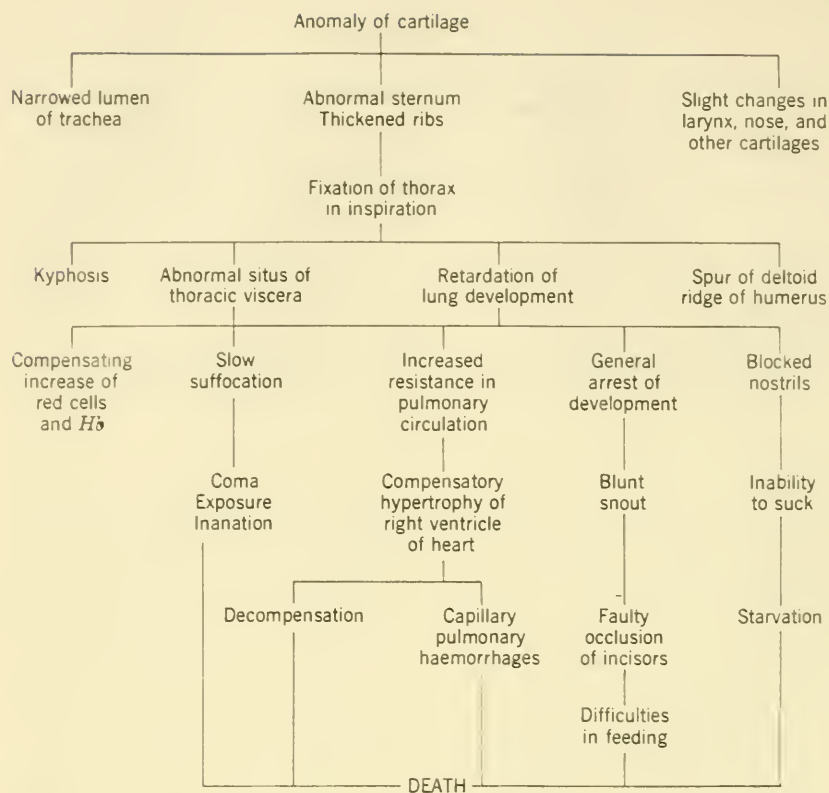


FIGURE 10.1. Pedigree of causes of death from a single lethal gene in the rat (from Grüneberg, 1947, *Animal Genetics and Medicine*, New York, Hoeber).

pigment is present in the flowers of BB and Bb plants, while in bb plants, the flowers are ivory or white. In the snapdragon, plants of the genetic constitution XY or Yy have yellow flowers containing two anthoxanthins, both of which are absent (or undetectable) in white-flowered plants which are yy .

In the primrose, evidently a single gene mutation has resulted in the alteration or loss of a single pigment. Specific biochemical differences between wild-type and mutant organisms have been demonstrated in many isolated instances in higher plants and animals. More frequently, however, the analysis has indicated that a single gene mutation has given rise to more than one change in the phenotype. This phenomenon, called *pleiotropism*, is a reflection of the highly integrated state of metabolic and developmental pathways. The snapdragon example, in

which two pigments are lost following a single gene mutation, represents the simplest sort of pleiotropism. At the other extreme, consider a lethal single gene mutation in the rat, cited by Grüneberg, in which the first observed effect is upon cartilage formation. The subsequent effects are so manifold and diverse that Grüneberg has provided a "pedigree of causes" of death, reproduced in Figure 10.1.

It is to be expected then, that the analysis of gene action will be greatly complicated by the integrated state of cellular and developmental metabolism, by the remoteness of the phenotype from the primary action of the gene, and by the number of intervening steps which can be influenced by other genes and by environmental factors. A fruitful approach would seem to require the choice of a trait directly determined by a gene, in which interactions with other systems are minimal.

The early studies of biochemical genetics (before 1940) played the important role of demonstrating in a few favorable systems, such as plant and animal pigments, that a specific gene mutation could lead to a block at a particular step in the biosynthesis of a particular compound. But investigators who attempted to carry this kind of analysis further in higher organisms found the going difficult.

A great advance in the search for systems suitable for the study of gene action came with the development of microbial genetics. In choosing the mold *Neurospora* for genetic investigation, Beadle and Tatum were motivated precisely by the hope that a microorganism would provide better access to the so-called primary gene products, the first chemical products of gene action, than could be expected with any higher organism.

GENETIC BLOCK OF SINGLE-STEP REACTIONS

Beadle and Tatum proposed the "one-gene-one-enzyme" hypothesis which states that a single gene acts by determining the specificity of a particular enzyme, "and thereby controls in a primary way enzymatic synthesis and other chemical reactions in the organism." On this hypothesis, preferred traits to be sought in the study of primary gene action are the presence, absence, or alteration of a particular enzyme following mutation.

It was expected that any enzymatically controlled reaction in the cell would be blocked by a mutation which interfered with the activity of the enzyme. On this hypothesis, then, to find the particular enzyme in each instance, it was necessary first to identify which biosynthetic step had been blocked as a result of a particular gene mutation.

Methods were developed, as mentioned first in Chapter 1 initially by Beadle and Tatum and then by many other investigators, for isolating mutant strains, now called *auxotrophs*, each of which, as the result of a single gene mutation, required a particular growth factor such as an amino acid, vitamin, purine, or pyrimidine, not required for growth by the wild-type strain. Large numbers of such mutants were found, and with many of them, the particular biosynthetic step blocked by mutation was identified. The use of these mutant strains greatly facilitated the analysis of the biosynthetic pathways of many of the amino acids, nucleotides, and cofactors of intermediary metabolism.

These investigations firmly established the principle that *gene mutations lead to blocks in specific step reactions*, and, by inference, that wild-type genes act by making possible those reactions which the mutant genes block. Similar proposals had been made numerous times in the past, for example, by the Dutch microbiologist Beyerinck in 1903, and the English physician Garrod in 1923. Nonetheless it is the studies carried out since 1941 with the auxotrophic mutants of *Neurospora*, *E. coli*, and other microorganisms that have provided a vast body of evidence indicating that *virtually all biosynthetic reactions which are under enzymatic control are thereby under gene control*.

QUALITATIVELY ALTERED ENZYMES

But what do these investigations tell us about the *mechanism* of gene control? There has been a widespread tendency to assume that, if a particular step is blocked following mutation, then, *a priori*, the enzyme mediating that step has been altered or lost. Upon further scrutiny, this assumption has proved to be invalid in many instances. The blocking of a reaction following mutation may result from (a) a qualitative or quantitative change in the enzyme controlling that reaction step, or (b) a change elsewhere which indirectly affects the reaction. Since branching and reversibility are common features of the pathways of intermediary metabolism, many of the observed blocks in particular reactions following mutation actually result from the indirect effects of other systems. In such instances, the enzyme of the blocked reaction may be quite normal. A number of such possibilities are indicated in Figure 10.2.

An example is the tyrosinase-forming system in *Neurospora*, briefly described in Chapter 1. Two different loci, *ty-1* and *ty-2*, have been identified, each of which totally blocks tyrosinase formation except under special nutritional conditions; then the enzyme is formed. Thus the identification of the step blocked by mutation does not provide an un-

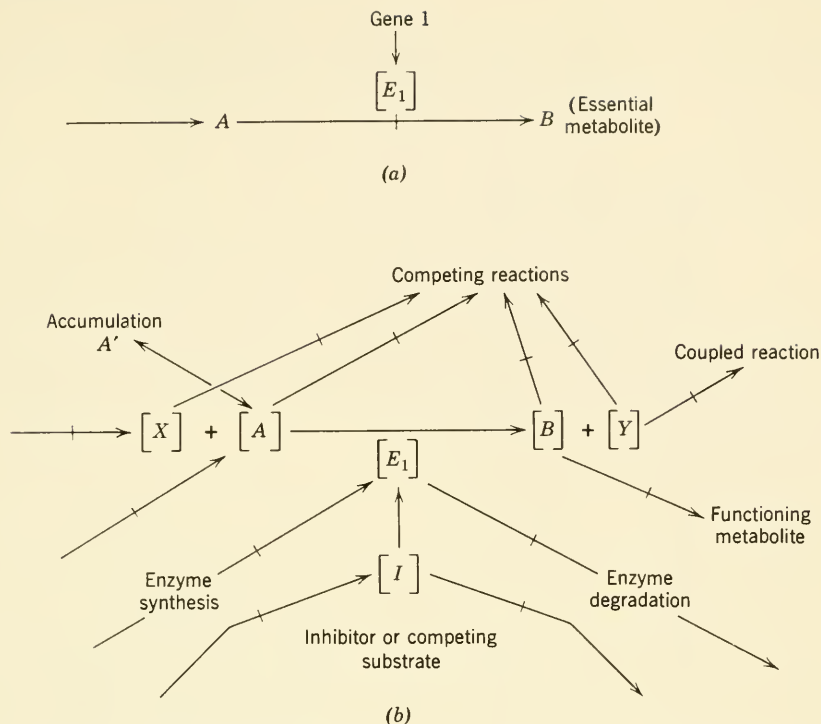


FIGURE 10.2. Diagram of a metabolic block in the conversion of A to B resulting from mutation.

(a). Simplified scheme. Mutation in gene 1 leads to inactivation of enzyme E_1 .

(b). Other possibilities. Inability to form B could result from a mutation altering any one of the reactions which influence the conversion of A to B (from Wagner & Mitchell, 1955, *Genetics and Metabolism*, New York, Wiley, p. 219).

equivocal guide to the identity of the relevant enzyme. And so even at the cellular level, the correlation of genotype and phenotype is made difficult by the highly integrated state of intracellular metabolism.

Nonetheless, altered enzymatic activity following mutation has been demonstrated for about 50 different enzymes in microorganisms. In most of these systems, only a quantitative decrease or loss of activity has been shown. One cannot safely infer a mechanism of gene action from these examples, because, as just discussed, decreased enzyme activity may occur for many reasons in addition to alteration of the specific enzyme in question. The only systems in which the action of the gene can be attributed unequivocally to a direct effect upon an enzyme are those in which the mutated strain produces a qualitatively altered enzyme,

the particular properties of which determine the observed mutant trait. A few such systems have been described, and they are listed in Table 10.1 together with a brief comparison of some of their properties.

TRYPTOPHANE SYNTHETASE

For purposes of illustration, we shall discuss some of the properties of the tryptophane synthetase system, for which there are extensive genetic and biochemical data, providing examples of most of the phenomena which have been found in other systems as well. A unique feature of this system, which makes it particularly useful for the present discussion, is that mutants affecting the same enzyme have been studied both in *Neurospora crassa* and in *E. coli*.

The enzyme tryptophane synthetase (previously called tryptophane desmolase, the gene symbol being *td*) catalyzes the condensation of serine with indole to form tryptophane, and mutants have been identified initially by their tryptophane requirement. Recently it has been shown that the enzyme catalyzes three related reactions: (1) indole + L-serine $\rightarrow$ tryptophane; (2) indole + triose phosphate $\rightleftharpoons$ indole-3-glycerol phosphate; and (3) indole-3-glycerol phosphate + L-serine $\rightarrow$ tryptophane + triose phosphate. In *Neurospora*, one enzyme catalyzes all three reactions, whereas in *E. coli*, the enzyme has been separated into two components, A and B. Of these, A is active in reaction 2 and B in reaction 1, but neither A nor B exhibits more than a small fraction of its activity in the absence of the other. It has been proposed that A contains the indole-glycerol-combining site, and B the serine-combining site of the enzyme.

The A and B fractions have been separated by column chromatography. When they are subsequently mixed, full enzymatic activity is restored. Altered A or B components which have been isolated from mutant strains of *E. coli* are tested for activity by mixing them with the complementary component from wild type in order to approximate optimal conditions. Antibodies prepared against the enzyme can effectively block its activity. For example, antibodies against component A block all three reactions, and anti-B antibodies block reactions 1 and 3 but not 2. The antibody against the *Neurospora* enzyme blocks all three reactions.

The complexity of this system provides a number of parameters for comparison of mutant enzymes with wild type, including enzyme activities in the three reactions, based on comparisons of rates, substrate affinities, pH optima, activation energies, and immunological cross reactivity.

TABLE 10.1
Enzymes Qualitatively Altered by Mutation

Enzyme	Organism	Reaction Involved	Enzyme Alteration
1. Tryptophane synthetase	<i>Neurospora</i>	(1) indole + serine $\rightarrow$ tryptophane (2) indole $\rightleftharpoons$ indole glycerol phosphate (3) indole glycerol phosphate + serine $\rightarrow$ tryptophane + triose phosphate same as above	<i>strain td-24</i> — increased metal sensitivity <i>strain td-2</i> — active only in reaction 2
2. Tryptophane synthetase	<i>E. coli</i>		<i>A mutants</i> — active only in reaction (2) <i>B mutants</i> — active only in reaction (1) increased thermolability
3. Pantothenic acid synthetase	<i>E. coli</i>	β -alanine + pantoic acid $\rightarrow$ pantothenic acid	increased thermolability mutant enzyme requires heat activation
4. Tyrosinase	<i>Neurospora</i>	oxidation of tyrosine	
5. Glutamic acid dehydrogenase	<i>Neurospora</i>	α -ketoglutarate + NH_4^+ $\rightarrow$ glutamate	
6. Pyrroline-5-carboxylate reductase	<i>Neurospora</i>	pyrroline-5-carboxylate $\rightarrow$ proline	increased thermolability and increased energy of activation
7. Alkaline phosphatase	<i>E. coli</i>	?	increased thermolability
8. Adenylosuccinase	<i>Neurospora</i>	AMP-succinate $\rightarrow$ AMP + fumarate	increased thermolability
9. Folic-acid-forming enzyme	<i>pneumococcus</i>	?	altered substrate affinities

Furthermore, mutant enzymes can be characterized by the amount of enzyme activity per cross-reacting unit.

In *Neurospora* 25 *td* mutants have been studied, all of them localized in one small region of the genetic map. None contain measurable amounts of the enzyme except the temperature-sensitive strain, *td*-24, which has been shown to contain an altered enzyme which is more sensitive to heavy metal inhibition than is the wild type. Some of the *td* mutants form a cross-reacting material (CRM) which is serologically related to the enzyme, and may represent it in an inactive form. Some mutants accumulate CRM and others do not; both classes are revertible to wild type and, therefore, do not represent chromosomal deletions. One of the CRM-forming mutants, *td*-2, contains an altered tryptophane synthetase which can carry out reaction 2 but not 1 or 3; thus it resembles the A component of the *E. coli* enzyme.

Some 60 ultraviolet-induced mutants of *E. coli* have been studied in which tryptophane synthetase activity is reduced or absent. Four classes are represented: those lacking A activity, those lacking B activity, and those with altered A or B components. Those mutants which totally lack A or B activity do not form any CRM, while those with altered activity contain either A-CRM or B-CRM respectively. These mutants have been mapped by transduction, and the A and B mutants have been located in separate but closely linked regions. Within each region, CRM-formers and CRM-nonformers are not separated from each other but rather they are interspersed along the map, as shown in Figure 10.3.

Of the 24 A mutants which have been studied, 19 are totally inactive, and 5 form an altered A protein which is effective in the indole $\rightarrow$ tryptophane reaction, when combined with normal B, but is inactive in the other two reactions. There are some differences among the five mutants with an altered A component. One of them forms less A protein and more B protein than the wild type, and another forms an A protein which is more acid-labile than is the normal A protein.

Among the B mutants, four CRM-producers have been studied which contain an altered B component. Three of them form a B which can only catalyze the indolephosphate $\rightleftharpoons$ indole reaction, but is as effective in this reaction as is the normal B, when compared in relation to antigenic specificity. In the fourth strain, the B component exhibits slightly more activity per unit of antigenic material than does the wild-type B.

These results presumably represent just a sampling of the kinds of altered enzymes to be found; no attempt has been made to exhaust the possibilities.

These observations on the tryptophane synthetase system, as well as the others summarized in Table 10.1, are sufficient to provide evidence

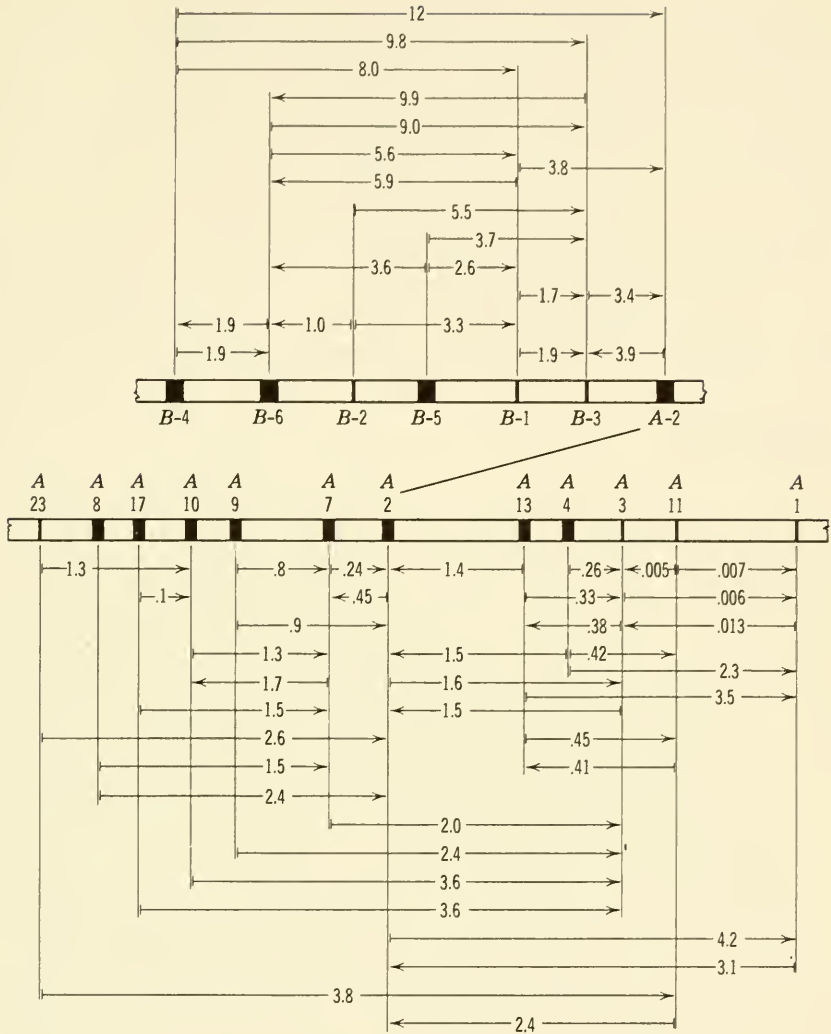


FIGURE 10.3. Genetic map of the tryptophane synthetase region of *E. coli*. Preliminary map of mutational sites for genes A and B. The black bars indicate the location of mutationally altered sites which lead to inability to form the A or B protein. The thin lines represent mutationally altered sites in mutants producing A-CRM or B-CRM. The values given were obtained in transduction experiments (the arrow indicates direction of transfer) and are based on the number of tryptophane-independent colonies which were observed relative to a control transduction from histidine dependence to independence (after Yanofsky, 1960, *Bact. Reviews*, **24**:221).

that the enzymes themselves have been altered, but they tell us very little about the precise chemical nature of the gene-induced alteration. Changes in thermostability, serological specificity, and catalytic activity all depend upon the folded three-dimensional configuration of the enzyme. Do these altered properties result from substitutions of one or more amino acids for others in the primary sequence of the protein? Many current investigations are designed to answer this question. As yet however, the relation between amino acid sequence and folded configuration is not well established, nor have analyses been reported for any of the enzymes listed in Table 10.1. Thus, it has not yet been shown in any instance that the observed differences in behavior of wild type and mutant enzymes result from altered amino acid sequences.

HEMOGLOBIN

One protein has been described, in which a single amino acid substitution is characteristic of a single mutant allele; that protein is hemoglobin. There is an altered form of hemoglobin, *HbS*, found in sickle cell anemia, a human ailment inherited as a single gene difference from normal. Sickle cell trait is a mild form of the disease, found in heterozygotes; individuals with sickle cell anemia are homozygous for the sickling gene. The anemia results from the presence of hemoglobin S (*HbS*) which tends to crystallize out in red blood cells at low oxygen tension, causing rupture of the cells. Heterozygotes contain, in each red blood cell, both *HbS* and normal hemoglobin, *HbA*, resulting in mild symptoms, if any.

The chemical distinction between *HbA* and *HbS* was first detected as a slight difference in electrophoretic mobility, that is, in their rate of movement in an electric field resulting from a net charge difference on the molecules. It was postulated that this charge difference resulted from a change in amino acid composition, but attempts to demonstrate such a change by analysis of total amino acid composition revealed no differences. A more sensitive method was needed.

The method that has worked so beautifully in the analysis of hemoglobin is called "fingerprinting," developed by Sanger for the sequence analysis of insulin. The protein is digested by trypsin, which breaks only those peptide bonds involving either lysine or arginine. The resulting peptides, most of which differ from one another in net charge, can be separated by paper electrophoresis, the peptides migrating in one dimension on wet filter paper. Subsequently, the paper is rotated through 90°,

and a further separation is elicited by different rates of migration of the peptides in particular solvents. The resulting two-dimensional separation consists of a distribution of spots, which can be visualized by staining with ninhydrin; an example is shown in Figure 10.4.

Hemoglobin contains 600 amino acids per molecule, arranged in four polypeptide chains, two identical α -chains and two identical β -chains. For purposes of amino acid analysis, one need consider only the 300 amino acids of the basic half-molecule. Comparison of the fingerprints of *HbA* and *HbS* indicated identity except for a single peptide, # 4. Total amino acid analysis of this peptide from *HbA* showed that, of the eight amino acids present, two were glutamic acid and one was valine, while the corresponding peptide from *HbS* contained one glutamic acid and two valines. The final step in analysis required determination of the precise sequence of the eight amino acids in peptide # 4. The results are shown in Table 10.2, together with the results of similar analyses undertaken subsequently with other abnormal hemoglobins. The second abnormal hemoglobin studied was *HbC*, in which a single amino acid, in this case lysine, was substituted for the same glutamic acid in peptide # 4. This result is particularly remarkable because genetic data indicate

TABLE 10.2
Chemical Differences in Hemoglobins
(From Hunt and Ingram, 1959, *Biochemistry of Human Genetics*,
Ciba Found. Symp., Little, Brown & Co., Boston, p. 114.)

<i>Hb</i>	Chain	Peptide	Amino Acid Sequence
<i>A</i>	β	4	Val . His ⁺ . Leu . Thr . Pro . Glu . Glu . Lys ⁺ . . .
<i>S</i>	β	4	Val . His ⁺ . Leu . Thr . Pro . Val . Glu . Lys ⁺ . . .
<i>C</i>	β	4	Val . His ⁺ . Leu . Thr . Pro . Lys ⁺ . Glu . Lys ⁺ . . .
<i>G</i>	β	4	Val . His ⁺ . Leu . Thr . Pro . Glu . Gly . Lys ⁺ . . .
<i>A</i>	β	26	. . . Val . Asp . Val . Asp . Glu . Val . Gly . Gly . - Glu . Ala . Leu . Gly . Arg ⁺ . . .
<i>E</i>	β	26	. . . Val . Asp . Val . Asp . Glu . Val . Gly . Gly . - Lys ⁺ . Ala . Leu . Gly . Arg ⁺ . . .
<i>D_β</i>	β	26	?
<i>L</i>	β	?	?
<i>D_α</i>	α	23	?
<i>I</i>	α	23	?
Hopkins-2	α	?	?
<i>Q</i>	α	?	?
<i>P</i>	α	?	?

Peptide #4 is a terminal peptide of the β -chain, peptide #26 is inside the β -chain.
. . . indicates end of peptide.

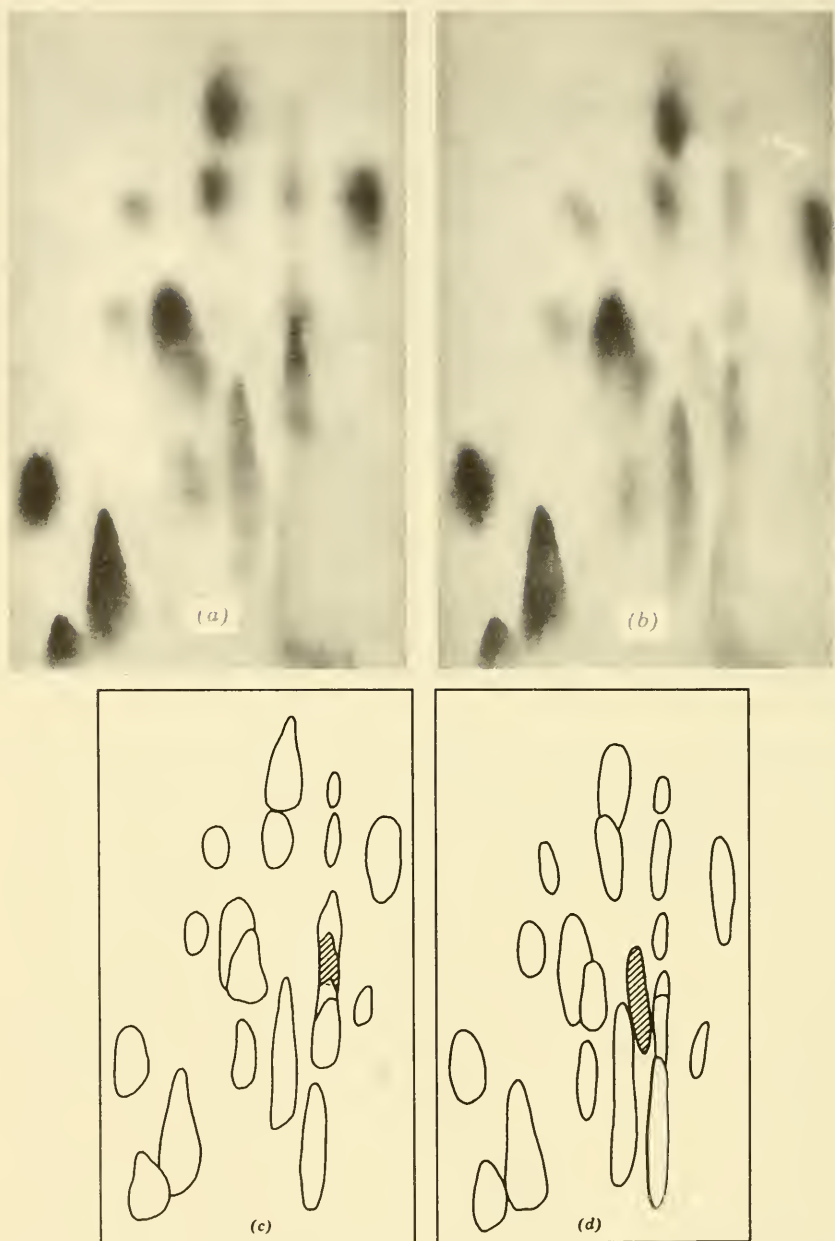


FIGURE 10.4. Hemoglobin fingerprint. Fingerprints made by a combination of electrophoresis and chromatography. (a) and (b) are photographs of normal hemoglobin (*HbA*) and sickle-cell hemoglobin (*HbS*) respectively. (c) and (d) are tracings in which the difference in pattern of spots is indicated by cross-hatching (after Ingram, 1958, *Scientific American*, January, p. 68).

no recombination between the factor determining sickle cell disease and that determining the particular anemia in which *HbC* is produced.

Thus gene mutation at a particular site in the DNA has resulted in single amino acid substitutions at a particular site in the protein. These findings support the view of a very precise relationship between mutationally altered DNA and altered protein. It should be noted, however, that the genetic data, coming from human pedigrees, are far less precise than are the chemical data.

Additional forms of hemoglobin are being reported at an accelerating rate, as improved methods for their detection become available. A number of them are listed in Table 10.2. Most of them were detected initially by altered electrophoretic mobility resulting from a charge difference. If one uncharged amino acid is substituted for another uncharged one, the altered protein may not be detected. Consequently, there may be many more forms of hemoglobin than are now known, and their recognition will depend upon a different principle of separation. The same problem applies to the detection of other altered proteins.

The dramatic clarity of the hemoglobin story supports the optimistic view that gene action may be a simple process in which genes determine amino acid sequence, and sequence in turn determines the folded, biologically active configuration of the protein. This view proposes the simplest possible solution to two outstandingly complex problems: gene action, and the relation of sequence to folding in protein structure. The very simplicity of this view recommends it. There are, however, numerous observations which do not fit well with this hypothesis. Before discussing them, we shall digress briefly to review some current concepts in the field of protein structure which are directly pertinent to our problem.

SOME CONCEPTS OF PROTEIN STRUCTURE

Proteins are large polymers built of approximately 20 different amino acids, held together by covalent peptide bonds. From the chemical point of view, there is essentially equal probability that any amino acid will be peptide-bonded with any other and, consequently, in a polymer of 100 amino acids, 20^{100} different arrangements are possible. Since cells contain only several thousand different proteins, it is evident that a very small group has been selected for biological activity from an almost infinite number of chemically possible molecules.

Many cellular proteins are very large, containing thousands of amino acids, and in their extended form encompassing lengths of several

thousand Ångströms. One would expect that they must be folded in some orderly way to avoid a tangled chaos within the cell, and this is indeed the case. The soluble proteins of the cells are approximately globular in form, as the result of folding of the polypeptide chains into specific three-dimensional configurations. These folded molecules exhibit the binding affinities, activities, and serological specificities which we measure. The folded configuration is presumably quite stable and uniform for each protein in any particular environment; physical and biochemical studies indicate considerable homogeneity.

What is known of the relationship between amino acid sequence and folding? In considering this problem, protein chemists have proposed the following useful terminology:

Primary structure refers to the order of peptide-bonded amino acid residues along the polypeptide chain. Some investigators include here the chemical cross links such as disulphide and phosphoester bridges between segments of the same or different polypeptide chains.

Secondary structure refers to the geometric arrangement of individual polypeptide chains, determined by the backbone peptide configuration and stabilized by interactions of the amino acid side chains. Best known is the α -helix described by Pauling *et al.*, and shown in Figure 10.5. A polypeptide in α -helical configuration is rather a rigid rod; bending can be occasioned by proline which does not fit into the tightly folded helix; and possibly by other bulky aromatic amino acids like tryptophane, phenylalanine, and tyrosine.

Tertiary structure refers to the final folded configuration of the protein including spatial relations between neighboring segments of the same or different polypeptide chains, which result from the combined force of many interactions between side chains, bonds involving metals such as zinc in insulin, electrostatic attractions, and various unspecified hydrogen bonds.

So far as is known, enzymatically active proteins are in a folded or "native" configuration. Consequently, the secondary and tertiary structures are most relevant to considerations of biological activity. Recently, the term *quaternary structure* has come into use to denote aggregated subunits, for example, the four polypeptide chains of hemoglobin. The state of aggregation may also influence biological activity.

Protein chemists are investigating the question: To what extent does the primary structure determine the folded configuration of the protein in any particular environment? With the development of new analytical techniques, it has become possible to determine the amino acid sequence of an entire protein, and thereby specify completely the primary struc-

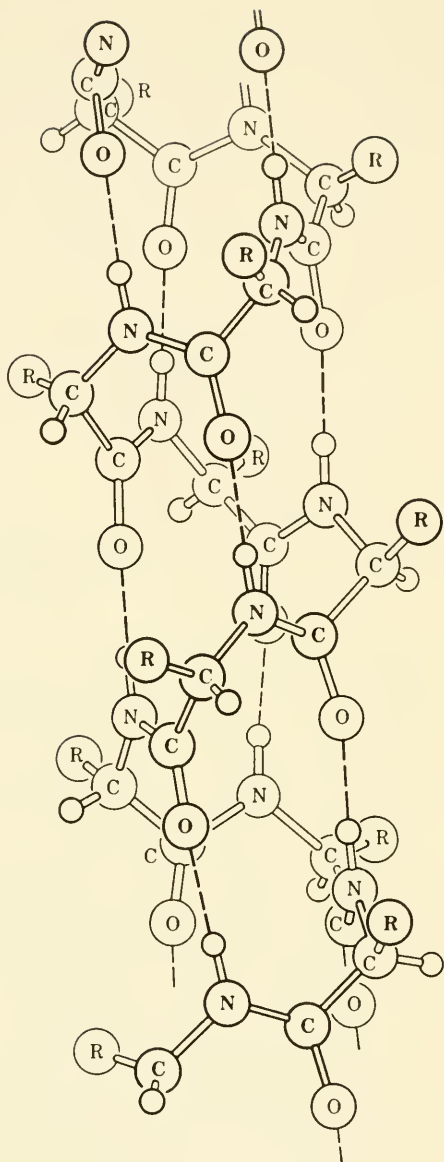


FIGURE 10.5. The α -helix configuration of a protein. The α -helix is held together by hydrogen bonds between the amide group of one amino acid and the carboxyl oxygen of the amino acid located three residues from it along the polypeptide chain. Formed in this way, the configuration is packed very firmly, and there is no open space in the center. The α -helix is a very general structure which will accommodate any amino acid side chains. Proline causes a bend in the chain (from Corey and Pauling, 1955, *Rend. Ist. Lombard Sci.*, **89**:10).

ture. This feat has already been achieved with a few small proteins including adrenocorticotrophic hormone (ACTH) (39 amino acids), insulin (51 amino acids), and ribonuclease (124 amino acids). The same methods can in principle be applied to all proteins, but, of course, the

come apart after cleavage of the peptide bond but remain associated, and the enzyme remains fully active. By mild acid treatment, the parts can be separated, and both of the fragments are enzymatically inactive. If these fragments are then mixed, they recombine in equimolar amounts to reconstitute the active enzyme. It is possible that some genetic control devices of the cell employ analogous mechanisms, in which the addition or removal of a short peptide sequence determines enzyme activity. Only isolation and analysis of the enzyme in question could test this proposal.

Information of the greatest importance about protein structure is coming from the incredibly complex and beautiful analysis of the tertiary structure of hemoglobin by means of X-ray crystallography. In these studies, atomic structure is observed by means of X-ray diffraction patterns which provide the raw data for calculating relative electron densi-

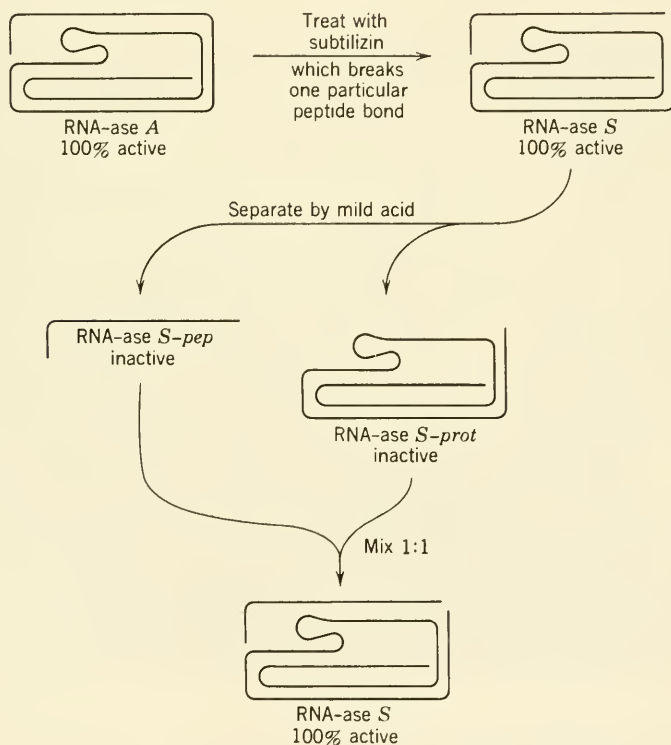


FIGURE 10.7. Spontaneous reassembly of two inactive fragments of ribonuclease restoring full enzymatic activity (after Richards, 1958, *Proc. Natl. Acad. Sc. Wash.*, 44:162).

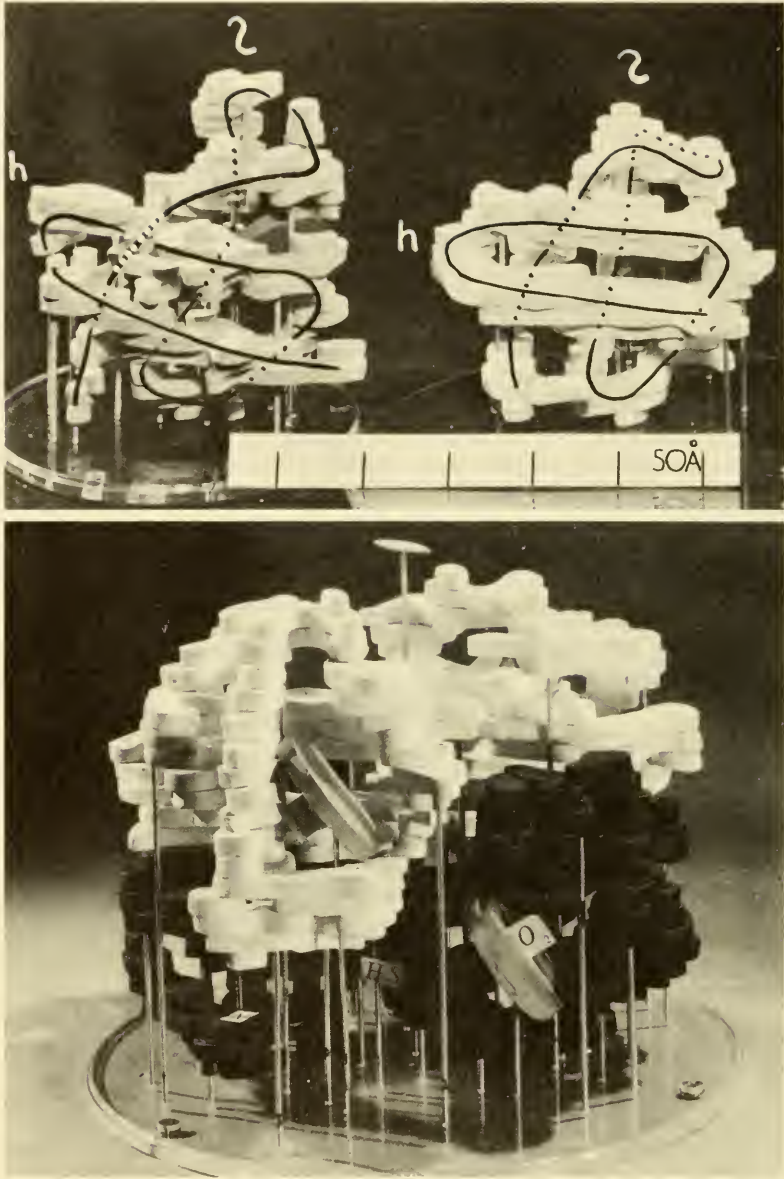


FIGURE 10.8. Models of the α - and β -polypeptide chains of hemoglobin (from Perutz et al., 1960, *Nature*, **185**:416).

Top. One each of the α - and of the β -chains, showing the path of the α -helix traced onto the model as a black solid and dotted line. The heme groups are at the backs of the chains and therefore not visible.

ties, and thereby the positions of the atoms. Because in hemoglobin the unit cell is some 40 atoms thick, the final two-dimensional picture which emerges from this analysis is too complicated to resolve in terms of a three-dimensional picture. Recently a new method, called isomorphous replacement, has been devised by Perutz at the Cavendish Laboratory in Cambridge, England, to meet this problem. The method compares the X-ray pattern of a crystalline protein with that obtained from the same protein after attachment to a specific site of a heavy atom, like gold or mercury, which shifts the pattern. By comparing diffraction patterns before and after replacement, certain data can be obtained which greatly simplify the analysis.

With this method, a group of investigators have been studying the α - and β -chains of hemoglobin and of the related protein myoglobin, which is an oxygen carrier in muscle and consists of just one polypeptide chain. Perutz and co-workers have established the tertiary configurations of the α - and β -chains of hemoglobin, based upon three-dimensional Fourier syntheses obtained by X-ray analysis at the 5.5 Å level of resolution. This magnificent feat of reconstruction of the hemoglobin molecule may be visualized by means of the models shown in Figure 10.8. The similarity in over-all structure of the α - and the β -chains is apparent from the models, and is particularly surprising since their amino acid compositions differ considerably, and the structure of the two chains is affected by different genetic loci (Table 10.2).

Kendrew and co-workers have established, by similar methods, the three-dimensional configuration of myoglobin at the 2.2 Å level of resolution. The myoglobins from different species have amino acid compositions which differ to some extent, sufficiently in sperm-whale and seal myoglobins to determine differences between them in crystal lattice. Nonetheless, the two-dimensional X-ray diffraction patterns of these two proteins are essentially identical. Also, the three-dimensional configurations of myoglobin and of the hemoglobin polypeptides are very similar. These are just a few points of special relevance to genetics which have already emerged from this remarkable series of investigations.

In considering the relation between amino acid composition and tertiary structure, one line of interpretation proposes that certain amino acids may be critical for determination of the folded configuration, such as proline for bends of the α -helix, and cysteine for the position of the

Bottom. The entire hemoglobin molecule consisting of two each of the α - and the β -chains assembled. Two heme groups can be seen lying in separate pockets on the surface of the molecule. O_2 indicates the face of the heme to which oxygen attaches. *HS* marks the position of one of the sulfhydryl groups. *N* denotes the *N*-terminal end of one of the polypeptide chains.

TABLE 10.3

**Comparison of Amino Acid Sequence of Cytochrome *c* Peptide to
which Heme Attaches**

Italicized amino acids are those which differ from species to species;
.... indicates end of the peptide.

(After Vaughan and Steinberg, 1959, *Adv. in Prot. Chem.*, **14**:140.)

Horse	 <i>Val</i> . <i>Glu(N)</i> . <i>Lys</i> . <i>Cys</i> . <i>Ala</i> . <i>Glu(N)</i> . <i>Cys</i> . <i>His</i> . <i>Thr</i> . <i>Val</i> . <i>Glu</i> . <i>Lys</i>
Beef	 <i>Lys</i> . <i>Cys</i> . <i>Ala</i> . <i>Glu(N)</i> . <i>Cys</i> . <i>His</i> . <i>Thr</i> . <i>Val</i> . <i>Glu</i> . <i>Lys</i>
Pig	 <i>Lys</i> . <i>Cys</i> . <i>Ala</i> . <i>Glu(N)</i> . <i>Cys</i> . <i>His</i> . <i>Thr</i> . <i>Val</i> . <i>Glu</i> . <i>Lys</i>
Salmon	 <i>Val</i> . <i>Glu(N)</i> . <i>Lys</i> . <i>Cys</i> . <i>Ala</i> . <i>Glu(N)</i> . <i>Cys</i> . <i>His</i> . <i>Thr</i> . <i>Val</i> . <i>Glu</i>
Chicken	 <i>Val</i> . <i>Glu(N)</i> . <i>Lys</i> . <i>Cys</i> . <i>Ser</i> . <i>Glu(N)</i> . <i>Cys</i> . <i>His</i> . <i>Thr</i> . <i>Val</i> . <i>Glu</i>
Silkworm	 <i>Val</i> . <i>Glu(N)</i> . <i>Arg</i> . <i>Cys</i> . <i>Ala</i> . <i>Glu(N)</i> . <i>Cys</i> . <i>His</i> . <i>Thr</i> . <i>Val</i> . <i>Glu</i>
Yeast	 <i>Phe</i> . <i>Lys</i> . <i>Thr</i> . <i>Arg</i> . <i>Cys</i> . <i>Glu</i> . <i>Leu</i> . <i>Cys</i> . <i>His</i> . <i>Thr</i> . <i>Val</i> . <i>Glu</i>

disulphide bridges. There may, however, be regions of the polypeptide chain in which the particular amino acids present are not of critical importance either for activity or for configuration, and these may be the ones which vary from species to species.

Another line of investigation concerns what is known as the "active site" of the enzyme. What chemical and structural alterations can occur without loss of enzymatic activity? Intensive investigations of a few enzymes have revealed the rather unexpected finding that considerable tampering with structure is possible in some instances without loss of activity. An extreme example is papain, which can be degraded by the action of the enzyme leucine aminopeptidase down to less than half of its original size without loss of activity. On the other hand, the slightest degradation of the peptide hormone glucagon leads to inactivation. In general, enzymes have been found to contain some regions which are much more sensitive than others to any structural change. Thus enzymes have "active sites" or catalytic regions to which the substrate is bound, and which are highly substrate-specific in their configuration. Other regions of the molecule may be less directly implicated in enzyme function, and structural alterations there may have less drastic effects upon function. For example, the enzyme β -galactosidase is not inactivated at all by combining with its specific antibody, although the combination with antibody is completely inactivating for most enzymes. Presumably the active site of β -galactosidase is exposed and available to substrate in the enzyme-antibody complex.

A beautiful example of the essentiality of some amino acids and not of others at a functionally important site is afforded by studies of cytochrome *c* from different species. The peptide to which the heme pro-

thetic group attaches has been analyzed for its amino acid composition and sequence; the results are summarized in Table 10.3.

In summary, it is not yet evident to what extent the primary structure of proteins uniquely determines their folded configuration, and thereby their functional properties. Thus, the geneticist cannot yet be assured that functional differences in enzymes extracted from wild-type and mutant cells result from substitutions of one amino acid for another. Nonetheless, amino acid substitution is clearly one mechanism by which the properties of a protein can be altered. For this reason alone, the hypothesis that mutations are expressed as altered amino acid sequences is a very appealing one. Let us turn now to data on the phenomenon of complementation and suppression which do not seem to fit so readily into this picture.

ALLELIC COMPLEMENTATION

Allelic complementation consists in the formation by heterokaryons or heterozygotes made from two allelic mutants of an active enzyme which neither produces alone. In the examples so far reported, the extent of enzyme activity is always far below that of the wild type. This phenomenon can be distinguished from the one discussed in Chapter 6, in which pairs of mutants are classified into different cistrons by the *cis-trans* test. There, full wild-type activity is exhibited when mutants from different cistrons are combined in the *trans* position.

Complementing allelic mutants have been reported at a number of loci in *Neurospora*, and it is beginning to look as if the phenomenon were very widespread. Initially, measurements were based upon restoration of growth but, for further analysis of the phenomenon, it is essential to study the enzyme itself; growth is an indirect and qualitative index of restored enzyme activity. Another important feature of complementation is that only a fraction of the mutants recovered are able to complement; this generalization is based upon reports of studies of about 15 different loci.

The most extensive study of complementation in relation to linkage within a locus has been reported for the *pan-2* region of *Neurospora* (Figure 10.9). A large number of mutants, both complementing and noncomplementing, have been mapped by recombination analysis, and the two types are found interspersed along the genetic map. Whatever determines ability or inability to complement is not localized in any particular region within the functional unit.

The complementing mutants can also be mapped in relation to one

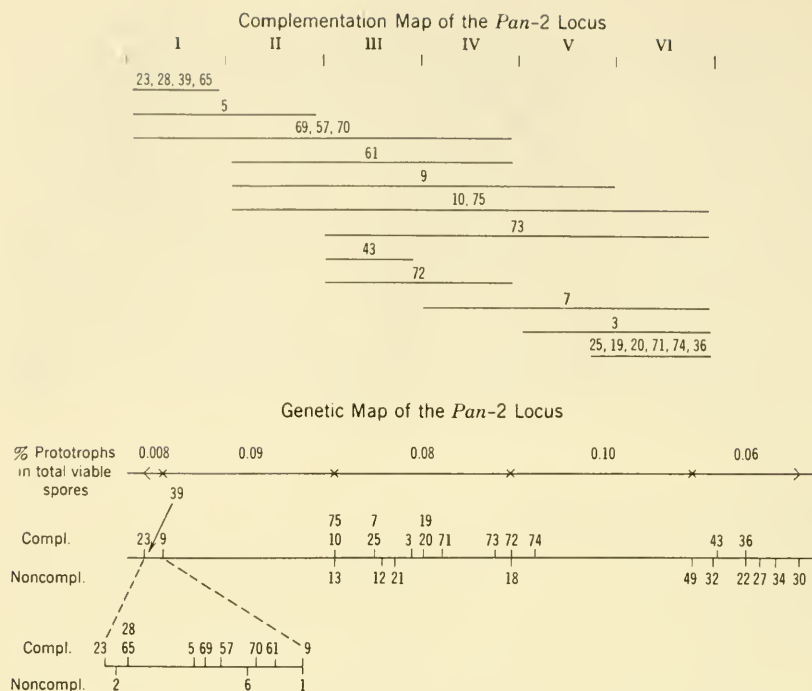
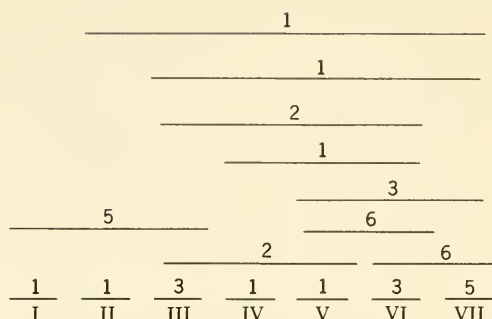


FIGURE 10.9. The *pan-2* locus of *Neurospora*. Independently isolated alleles at the *pan-2* locus are denoted by number, and their location is shown both on the complementation map and on the genetic map of the region. Complementing alleles are shown above the map line, noncomplementing alleles below the line (after Case and Giles, 1960, *Proc. Natl. Acad. Sci. Wash.*, **46**:659).

another in the form of a complementation map, which is in principle the same as a deletion map (Fig. 6.6). In complementation, however, the damage is not a deletion, since these mutants revert to wild type. One assumes that noncomplementation results from the presence of damage in identical regions of the two mutants in question, and complementation from nonoverlap of damaged regions. Mapping then consists in arranging all mutant pairs according to these criteria. Surprisingly, when this procedure was followed with the *pan-2* mutants (and with those at other loci as well), a linear map was produced, and the order correlated to some extent with that of the genetic map. The genetic data have not been correlated with enzyme studies at the *pan-2* locus, but this has been done with the *ad₄* locus.

Mutants at the *ad₄* locus lack the enzyme adenylosuccinase (see Table 10.1), and heterokaryons composed of complementing alleles show 2 to 25 per cent of wild-type activity. An extensive complementation map

FIGURE 10.10. The ad_4 locus of *Neurospora*. Complementation map of the ad_4 locus based on both primary and secondary mutants. Roman numerals denote seven distinct functional regions. The figure above each line denotes the number of independent ad_4 mutants in each category (from Giles, 1959, *Proc. X Inter. Congr. Genetics*, Montreal, 1958, 1:261).



exists for this locus, as shown in Figure 10.10, but only a poor genetic map is available owing to the infertility of crosses. Those mutant strains which have been mapped indicate the same correlation between complementation map and genetic map seen at the *pan-2* locus. Because of the availability of an enzyme assay method, the extent of restoration of activity by various pairs of mutants could be determined. As indicated in Figure 10.11, a correlation was observed between enzyme activity, and the map distance between members of complementing pairs of mutants. In this material, more distant alleles complement more effectively than do close ones.

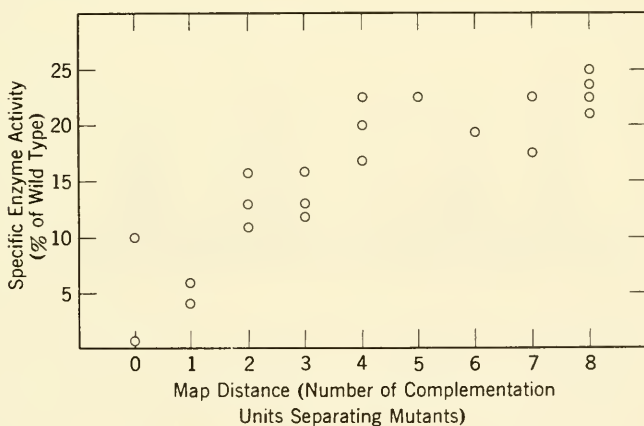
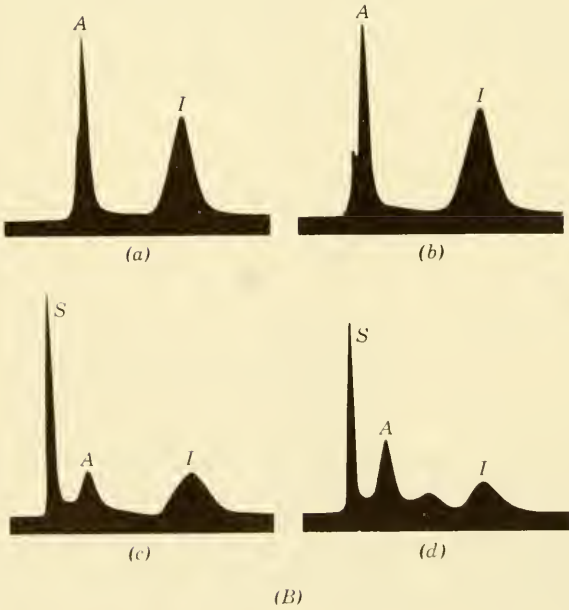
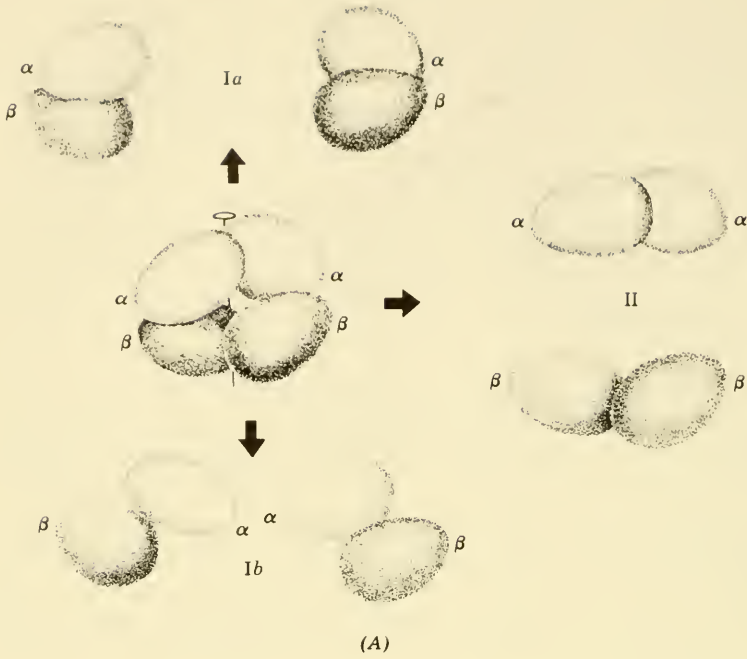


FIGURE 10.11. Complementation and enzyme activity at the ad_4 locus. Relationship between complementation map distance and adenylosuccinase activity in heterokaryons. Each point represents a heterokaryon composed of different pairwise combinations of adenylosuccinaseless mutants. Many of these figures are averages from several assays of separately grown cultures of the heterokaryons. The data show a definite relationship in which the enzyme activity of the heterokaryons increases with increasing map distance between the complementing alleles (after Woodward, 1959, *Proc. Natl. Acad. Sci. Wash.*, 45:846).



In further studies of the ad_4 mutants, *in vitro* complementation was discovered. By freezing and grinding together the separately grown mycelia of a pair of complementing strains, some enzyme activity was recovered in the homogenate. The occurrence of *in vitro* complementation raises more questions than it answers. The process probably involves gene products rather than the genes themselves, since it occurs in the structurally disorganized mycelial homogenate. However, one can only speculate about this at present, for no evidence is yet available concerning the mechanism of the process.

A possible mechanism of complementation has been proposed by Singer and Itano, who have been studying *in vitro* recombination between the polypeptide chains of hemoglobin. They found that the four polypeptide chains could be dissociated asymetrically, the two α -chains separating from the two β -chains, as shown in Figure 10.12, and that subsequently these nonidentical halves could be induced to reassociate.

The reassociation process has been investigated by labeling the polypeptide chains in a number of different ways. Here we shall limit ourselves to a single example, in which genetically altered hemoglobins were employed. The chemist's terminology and the net charge differences of these hemoglobins are:

<i>HbA</i>	$\alpha_2^A \beta_2^A$	Neutral
<i>HbS</i>	$\alpha_2^A \beta_2^S$	2
<i>HbI</i>	$\alpha_2^I \beta_2^A$	-4

The superscript A refers to normal hemoglobin, and the other superscripts refer to the abnormal types; this notation scheme makes clear in which chain the abnormality is located.

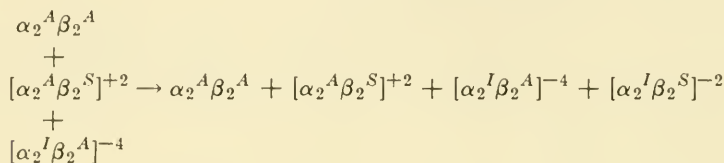
Because of the charge differences between these three types of hemoglobin, they can be separated from one another by electrophoresis. In

FIGURE 10.12. Recombination of hemoglobin polypeptides (after Itano, 1960, *Acad. Sci., New York*, **88**:642).

A. The possible types of dissociation of hemoglobin subunits are three: symmetrical dissociation as in types Ia and Ib, and asymmetrical dissociation as in type II. Under the conditions of this experiment, only type-II dissociation occurred, as demonstrated below

B. Electrophoretic diagrams showing the charge distribution of hemoglobin proteins. (a). A mixture of *HbA* and *HbI* which differ by four net charges. (b). The same mixture after dissociation and reassociation, showing that only type-II dissociation occurred. (c). A mixture of *HbA*, *HbS*, and *HbI* before dissociation. (d). The same mixture after reassociation, showing an increase in *HbA*, a decrease in *HbI*, and the presence of a new *Hb* with a net charge of -2. These changes result from random recombination of α -chains with β -chains following asymmetrical dissociation (type II).

Figures 10.12 *a* and 10.12 *b* a mixture of *HbA* and *HbI* is shown before and after reassociation. There has been no change in charge distribution, since either pair of α -chains combined with either pair of β -chains will produce only the same two types of molecules as were initially present. When *HbS* is added to this mixture, however, as in Figures 10.12 *c* and Figures 10.12 *d*, a new component appears, and there is an increase in the *HbA* peak. The equation describing the recombination is written:



There are many interesting applications of these experiments, among them, a new method for determining which chain of an unknown hemoglobin contains the abnormality responsible for a net charge difference from *HbA*. With respect to complementation, the significant point is the ability of a protein to undergo recombination of its constituent polypeptide chains under mild, virtually physiological, conditions.

Might complementation also result from recombination of polypeptide chains? In the tryptophan synthetase system of *E. coli*, any mutant *A* will complement with any mutant *B*, in tests carried out by means of abortive transduction. This result is not surprising, since *in vitro* studies of the *A* and *B* components demonstrated that they could be separated and recombined without loss of activity. In this system, complementation may well result from a mechanism of recombination analogous to that of hemoglobin. A closer comparison with the *Neurospora* complementation systems would be afforded by studies of complementation within the *A* and *B* regions of *E. coli*, but such studies have not yet been reported.

In the *ad*₄ region of *Neurospora*, ten complementation units have been described. If each gives rise to a different polypeptide, then in the heterokaryon, normal units could be substituted for mutant ones, reconstituting active enzyme. There are many difficulties with this interpretation, among them the fact that the linearity of the complementation map remains unexplained, as does the greater efficiency of complementation with increased map distance between mutants. Another proposal considers complementation as the aggregation of identical units, with two inactive enzymes aggregating to form one active unit. On this view, the *ad*₄ region is all one cistron making one polypeptide chain,

and the complementation units reflect the relative ease or difficulty of aggregation of these chains as a function of distance between the altered sites.

The experiments required to test these proposals have not yet been reported. If complementation does occur by reassortment or aggregation of polypeptide chains, then physical studies of the enzymes should reveal this. On the other hand, none of the proposed mechanisms accounts for all the biological observations, and it seems likely that further analysis of complementation may provide some new insights into the mechanism of gene action.

SUPPRESSION

Another phenomenon, which may in some instances result from the same process as complementation, is called suppression. The term is a poorly chosen and misleading one, but since it is in current usage, we shall employ it here. Geneticists use the term suppression to refer to a change in a mutant phenotype in the direction of the wild type following mutation at some second locus, not the site of the primary mutation. Thus, a suppressor mutation reverses the phenotypic effect of a primary mutation. Phenotypically, it resembles a reversion, and can be distinguished from it often by slow growth but definitively by genetic analysis to determine whether one locus or two are involved.

There may be many different biochemical mechanisms leading to reversal of the mutant phenotype. Three such mechanisms have been demonstrated: (1) synthesis of the required compound by an alternate pathway, thereby avoiding the blocked step; (2) removal of an inhibitor present in the primary mutant strain; and (3) formation of an active enzyme. Of these, the third is particularly interesting for the analysis of primary gene action, and it may be related in mechanism to complementation.

The tryptophane synthetase system provides examples of mechanisms 2 and 3. In *Neurospora*, strain *td-24* is temperature-sensitive, having an absolute growth requirement for tryptophane at 25°C but not at 30°C. At both temperatures, cultures form large amounts of CRM. Two different unlinked genes, *su-24* and *su-3*, are each able to restore some enzyme activity. CRM isolated from cultures of *td-24* grown at either temperature can be restored to enzymatic activity *in vitro* by removing a bound inhibitor. This activated enzyme from *td-24* differs from the wild-type enzyme in being more sensitive to metal ion inactivation. The enzyme isolated from suppressed strains *td-24 su-24* or from

td-24 su-3 has the same metal sensitivity as that of the mutant enzyme from *td-24*. This example shows that the *td-24* mutant contains an altered enzyme in respect to metal sensitivity, but that the enzyme can be activated by: (a) increasing the temperature at which the strain is grown; (b) purifying the enzyme *in vitro*; and (c) the occurrence of suppressor mutations. The fact that the enzyme in *td-24 su-24* resembles that of *td-24* and not of wild type indicates that the suppression involves removal of an inhibitor or some other indirect effect, but not a change in the enzyme.

Suppressors have been found for some of the tryptophane synthetase mutants of *E. coli* in which there do seem to be changes in the enzyme itself. For instance, the A-2 mutant does not produce any A-CRM, but the suppressed strain, A-2 *su-2*, forms an A protein indistinguishable from wild-type A in its catalytic action, but differing in its binding with the B component. Immunological tests revealed little difference between the A protein of the suppressed mutant and wild type. Another suppressed mutant, A-3 *su-3*, derived from the A-CRM-former, A-3, contains an A component with catalytic activities different from the wild-type A. By purification and fractionation it was shown that the suppressed strain contained two separable A components, one like wild-type A and the other like the A-CRM of the original A-3 mutant. In this example, it is clear that the suppressor mutation has resulted in production of active enzyme not previously present. However, the suppressor gene is not simply a duplication, since it acts only in the A-3 strain and not in other A mutants. Perhaps it is a duplication for only a part of the gene.

The occurrence of *in vitro* complementation led to the suggestion that the mechanism involves interactions of the enzymes or the enzyme-forming systems, but not of the genes themselves. This view is reinforced by the observations of suppression just discussed. Further support for this view comes from the studies of nonchromosomal male sterility discussed in Chapter 9. As may be recalled, there are at least two such factors of independent origin in maize, and a number of chromosomal genes which can partially or fully restore fertility in the presence of the sterility factors. The genes are factor-specific, in the sense that a particular gene can suppress a particular factor for male sterility and not others. This system is quite analogous to the allele-specific suppressors of tryptophane synthetase mutants, except that the male sterility determinants are nonchromosomal, while the genes which interact with them to restore fertility are chromosomal. If there is a unitary explanation for all these observations, it most likely will involve some steps in the process of enzyme formation, or more generally, of protein synthesis.

DNA BASE RATIOS AND GENE ACTION

The unitary hypothesis of gene action proposes that genes control cellular functions by determining protein specificity, and that this, in turn, is accomplished by the translation of a linear code from the nucleotide sequence of DNA to the amino acid sequence of protein. Recent studies of the physical properties of DNA extracted from a large number of different species have provided data not easily reconciled with the unitary hypothesis.

A method for determining the density of DNA by equilibrium sedimentation in cesium chloride was described in Chapter 7. When DNA extracts from different organisms were centrifuged by this method, it was discovered that they varied considerably in density. Of the four nitrogen

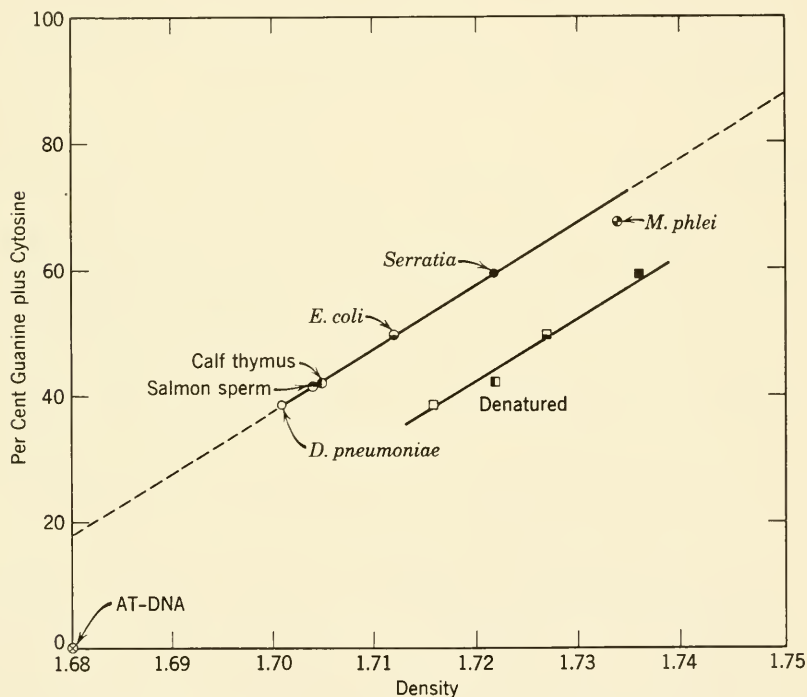


FIGURE 10.13. Relationship of density to the guanine-cytosine content of various samples of DNA. The density as determined by equilibrium centrifugation in cesium chloride (see Chapter 7) was correlated with base composition of both native and denatured samples of the DNA of a number of species (from Doty, Marmur, and Sueoka, 1959, *Brookhaven Symp. Biology*, 12:10).

bases in DNA, guanine is the heaviest, and so it was postulated that the densities might correlate with guanine content. An excellent correlation was found, as shown in Figure 10.13. Subsequent analyses of the sedimentation data demonstrate very little density heterogeneity among the molecules. Consequently, it may be inferred that virtually every DNA molecule of *Mycobacterium*, for example, contains more guanine than any of the DNA molecules from *E. coli*.

On the unitary hypothesis, one would expect considerable similarity in base ratios of DNA molecules from different species, since by and large they contain so many enzymes which are functionally identical and structurally very similar. A number of interpretations have been proposed to account for these findings, among them: (1) there is some species-specific noncoding DNA in every molecule; (2) that the code differs from species to species; (3) that the DNA codes only a small species-specific part of the protein molecule, the rest being coded by RNA; and (4) that the code is based on a two-letter alphabet in which the 6-keto-containing bases, guanine and thymine, are equivalent, as are the 6-amino compounds, adenine and cytosine. This is an unresolved question.

RESUMÉ

In this chapter we have focused attention upon the selection of particular traits well suited to an intensive analysis of gene action. The process of selection included: first, the choice of a microorganism instead of a multitissued plant or animal; second, the selection of a particular class of mutants, the auxotrophs, blocked at a particular step in some biosynthetic pathway; and third, the selection of mutant strains containing altered enzymes differing from the wild type in a manner accounting for the phenotypic difference between strains. With the aid of these criteria, about a dozen systems have been found in which the mode of gene action involves an influence upon protein specificity.

We must now retrace our steps to inquire how special or how general these selected systems may be. In addition to the few mutants in which altered enzymes have been identified, there are about three to four times as many mutants in which the absence of an enzyme corresponding to the blocked step has been reported. These instances are somewhat ambiguous, for the effect of the mutation may be to interfere with enzyme formation, or to inactivate the formed enzyme, or to alter its specificity so greatly that it is unrecognized. It is evident then, that, even in the best analyzed material, there is still a serious question about the precise *mechanism* of gene action. Nonetheless, it does appear likely that the

genes which have been studied act principally by their influence upon enzymes. Even in mutants in which the enzyme governing the blocked reaction is present, the block may result indirectly from the inactivity of a different enzyme.

A more general problem concerns the auxotrophic mutants themselves. What fraction of all possible mutations involve blocks in particular steps in biosynthesis? Attempts to answer this question experimentally have involved what are called "irreparable mutants," those which cannot be kept alive by any known growth factor supplemented in the medium. The frequency of this class in *Neurospora* has been estimated by two different methods.

One method, devised by Horowitz, employed temperature-sensitive mutants, a class in which the mutant phenotype is fully expressed only within a particular temperature range. The frequency of "irreparable" mutants was estimated by obtaining a number of mutants which would grow on minimal medium at 25°C but not at 35°C. Some of these, the "reparable" ones, would grow at 35°C on a supplemented medium, whereas others, which would not grow on any known combination of growth factors, were classed as "irreparable." In *Neurospora*, the irreparable class amounted to 46 per cent of the temperature-sensitive mutants recovered. (In *E. coli*, 23 per cent of the temperature mutants were irreparable, but the selection method is open to criticism and will not be considered further here.) Although temperature mutants are not rare among the auxotrophs, their use in this experiment is based upon the assumption, stated by Horowitz, that "genes controlling indispensable functions are just as likely to yield temperature-sensitive alleles as those controlling dispensable functions." This assumption is essentially untestable, but if valid, it would appear that at least half of the *Neurospora* auxotrophs recovered after UV-irradiation result from some unknown metabolic effect of the mutation.

The other method of estimating the frequency of irreparable mutations, devised by Atwood, utilized *Neurospora* heterokaryons composed of two mutant strains, and thus consisting of two types of nuclei within a common cytoplasm. Irreparable mutants are defined as those which can be kept growing within the heterokaryon by the functioning of the other nuclear component, but cannot grow when isolated in a monokaryon. The method developed to detect these mutants is extremely ingenious, can be applied to both induced and spontaneous mutations, and makes possible a very different basis for estimating indispensability than does the temperature-sensitivity method. Of the UV-induced mutants recovered by this method, over 96 per cent were found to be irreparable, and of the spontaneous mutants, about 92 per cent. All of these mutants,

furthermore, were shown to be nonallelic and, therefore, not repeats of the same mutation. The temperature-sensitive mutants of Horowitz were not checked for allelism.

Both methods provide estimates of irreparability, and both involve techniques and assumptions which are open to criticism. The impressive finding, however, is not the discrepancy between 50 per cent in one experiment and 90 per cent in the other, but rather the agreement between the methods in demonstrating that the majority of mutations, spontaneous and UV-induced, arising in *Neurospora* have never been studied, and may represent a reservoir of unknown gene functions.

These findings are very much in line with the results of classical mutation rate studies in *Drosophila*, in which the frequencies of lethal and of visible mutations were compared. The quantitative measure of mutation in *Drosophila* began with Muller's invention of the CLB method for counting the numbers of recessive lethals on the X chromosome. This and another method, using attached-X stocks, can also be employed for estimating the frequency of visible mutations. In comparative studies, the frequency of lethals was found to be about double that of visible mutations, following X-radiation. The spontaneous frequencies are too low to be studied by these methods.

It should be quite clear that the nature of "lethals" in *Drosophila* may have nothing more in common with the "irreparables" in *Neurospora* than that both represent unknown gene functions, accounting for the majority of detected mutations. In considering gene functions in an animal like *Drosophila*, one must take into account the complexities of embryological differentiation and the mechanisms required for integration of organs and tissues. We are hard put even to identify gene functions at the cellular level. There at least one may make a beginning and set up hypotheses susceptible to test.

Some suggestions have been made concerning the nature of "irreparable" mutations in *Neurospora*. For example, some may represent effects upon enzymes which catalyze reactions of very labile substrates or complexes which cannot be supplied readily from the medium. Other mutations may alter permeability; one such mutant in *Neurospora*, called osmotic, cannot tolerate media of high osmolarity. Some mutations may be lethal because precursors accumulate behind the blocked reaction and interfere directly or indirectly with growth. Clearly, with a little ingenuity, one may compile long lists of possible modes of lethality at the cellular level; for many of them there are examples on record.

These suggestions all refer to mutant effects depending ultimately upon the cell's ability to synthesize a particular enzyme. Is there evidence

of other modes of gene action? In Chapter 9, we have discussed the likelihood that determinants exist which influence pattern and organization of such structures as chloroplasts, mitochondria, and kinetosomes, and perhaps also of other structural components. Furthermore, the genetic identification of nonchromosomal determinants apparently unlinked with any known structures suggests the existence of virtually unexplored aspects of cell heredity.

Another line of evidence, based upon the nonrandom distribution of known genes, suggests that the genetic potentialities of the chromosomes themselves have not been fully explored. The organism in which the largest number of mutants has been identified is *Drosophila melanogaster*, in which even lethals have been mapped, and the genetic data have been correlated with cytological maps of the salivary chromosomes. Even in this organism, there are sizable regions, scattered throughout the genome, and no different in appearance from the rest, in which no mutants have been found. Sampling error cannot be invoked to explain these results, in view of the large number of mutants which have been obtained by all sorts of treatments. Similar evidence comes from the linkage maps of maize, which also show the nonrandom distribution of markers, and from *Neurospora*, in which no mutants have yet been found on 5 of the 14 chromosome arms.

In recent experiments with bacterial systems, a class of mutations was identified which appears to control only the rate of enzyme formation, not specificity. From these and related studies, a new dimension of gene action, often suggested from previous work with higher plants and animals, is becoming available for more critical analysis at the cellular level. This new information will be discussed in the next chapter, and then we shall return to a further consideration of gene action.

BIBLIOGRAPHY

- Atwood, K. C., and F. Mukai, 1953. Indispensable gene functions in *Neurospora*. *Proc. Natl. Acad. Sci. Wash.*, **39**:1027.
- Bonner, D. M., 1951. Gene-enzyme relationships in *Neurospora*. *C. S. H. Symp. Quant. Biol.*, **16**:143-157.
- Case, M. E., and N. H. Giles, 1960. Comparative complementation and genetic maps of *pan-2* locus in *Neurospora crassa*. *Proc. Natl. Acad. Sci. Wash.*, **46**:659-676.
- Catcheside, D. G., 1960. Relation of genotype to enzyme content. In: *Microbial Genetics*, 10th Symp. of the Soc. for General Microbiology. Cambridge, The University Press, pp. 181-207.

- Fineham, J. R. S., 1959. The biochemistry of genetic factors. *Ann. Rev. Biochem.*, **28**:343-364.
- Horowitz, N. H., and U. Leupold, 1951. Some recent studies bearing on the one gene-one enzyme hypothesis. *C. S. H. Symp. Quant. Biol.*, **16**:65-74.
- Linderstrom-Lang, K. U., and J. A. Schellman, 1959. Protein structure and enzyme activity. In: Boyer, P. D., H. Lardy, and K. Myrbäck (eds.). *The Enzymes* (2nd ed.), Vol. 1. New York, Academic Press, pp. 443-510.
- Perutz, M. F., 1958. Some recent advances in molecular biology. *Endeavor*, **17**:190-203.
- Perutz, M. F., M. G. Rossmann, A. F. Cullis, H. Muirhead, and G. Will, 1960. Structure of hemoglobin—a three-dimensional Fourier synthesis at 5.5Å resolution obtained by X-ray analysis. *Nature*, **185**:416.
- Sanger, F., and L. F. Smith, 1957. The structure of insulin. *Endeavor*, **16**: 48-53.
- Wagner, R. P., and H. K. Mitchell, 1955. *Genetics and Metabolism*. New York, Wiley.
- Wolstenholme, G. E. W., and C. M. O'Connor, 1959. *Biochemistry of Human Genetics* (CIBA Foundation Symp.). Boston, Little Brown & Co. (Especially articles by Itano, Singer, and Robinson; Hunt and Ingram; Brenner.)
- Yanofsky, C., 1960. The tryptophan synthetase system. *Bact. Rev.*, **24**:221-245.
- Yanofsky, C., and P. St. Lawrence, 1960. Gene action. *Ann. Rev. Microbiol.*, **14**:311-334.

11 Genetic Control of Cell Integration

Cell integration refers to the coordination of the metabolic processes proceeding within the structural framework of the cell. In actuality, integrative mechanisms encompass all of cellular biology. Students of heredity seek to define the position of the genetic determinants in these integrative mechanisms. In the preceding chapter we discussed one aspect of this problem: the genetic control of protein structure. This is in a sense a *static* aspect of gene function, for it concerns the presence or absence of a particular protein. If the only (or principal) contribution of the genetic material were to the determination of protein specificity, then the entire function of integration, the *dynamics* of cell biology, would be left to gene products and other nongenetic elements.

Many diverse lines of research are providing evidence that genetic elements are intimately involved in the dynamic features of intracellular transactions, but in our view, any attempt at a systematic discussion of the subject would be premature. Consequently, the purpose of this chapter is not to develop any over-all schema but rather to acquaint the reader with a few of the current directions of research which appear to be particularly revealing. Much of the work to be discussed in this chapter is still in progress, and the results must be considered preliminary. The importance of the researches to be discussed lies not only in the new "facts," which may not all stand the test of time but also, and perhaps more significantly, in the new lines of experimental attack being developed, and in the sharpening of concepts.

Traditionally, this area of research was catalogued under some such heading as "nucleocytoplasmic interactions," and the early work was carried out primarily with large and complex organisms. One of the early attempts to study the problem at the cellular level was made by Hämmerling, who utilized the enormous single-celled alga, *Acetabularia*.

This organism consists of a basal rhizoid in which the nucleus resides, a narrow stalk some 4 to 6 centimeters long, and a terminal cap of distinctive morphology which differs from species to species. By cutting the stalk, one obtains nucleated and anucleated portions. Hämmerling studied the regenerative capacities of these fragments, and he made interspecies graft hybrids with them. He found that segments of anucleated stalk were capable of regenerating new caps for a limited period, but that only nucleated regions could continue to do so repeatedly and indefinitely. Apparently, morphogenetic activity was retained by anucleated regions for a considerable time but, eventually, nuclear activity was required. In graft hybrids, as shown schematically in Figure 11.1, cap morphology was eventually determined by the nucleus, and if nuclei of both species were present, the cap form was intermediate.

These studies are of great interest as a pioneering attempt, and serve to illuminate some of the difficulties of the problem. The distinction between nucleate and anucleate involves far too many parameters to provide much analytical power. Similarly, the dichotomy between nuclear and cytoplasmic function does not help us very much; consider simply that the nucleus contains not only the chromosomes but also the nucleolus and the nuclear membrane, the outer layer of which is coated with cytoplasmic particles (ribosomes). This level of analysis is too general: more incisive methods are needed for a more penetrating analysis.

It is not an easy matter to study integrative mechanisms, which are, by their very nature, subject to disruption in the process of their analysis. Nevertheless, the investigation of biochemical pathways of synthesis of the macromolecular nucleic acids and proteins, as well as the cytochemical localization of these processes in specific regions and organelles of the cell, is providing an essential framework.

This chapter might appropriately be subtitled, "Life Among the Macromolecules," for the central focus of interest is upon genetic mechanisms controlling the formation and activity of the nucleic acids and proteins. Within the limits of our present understanding, cell integration at the molecular level may be thought of in terms of specificity and timing of synthesis of these macromolecules. On the simplest view, all other cellular features may be derived from these.

This formulation totally omits the question of whether subcellular structures, such as chloroplasts, mitochondria, and kinetosomes, are copied on a supertemplate in which structural specificity is conserved during replication in some novel manner. The information available on this problem has been summarized in Chapter 9. In this chapter, we

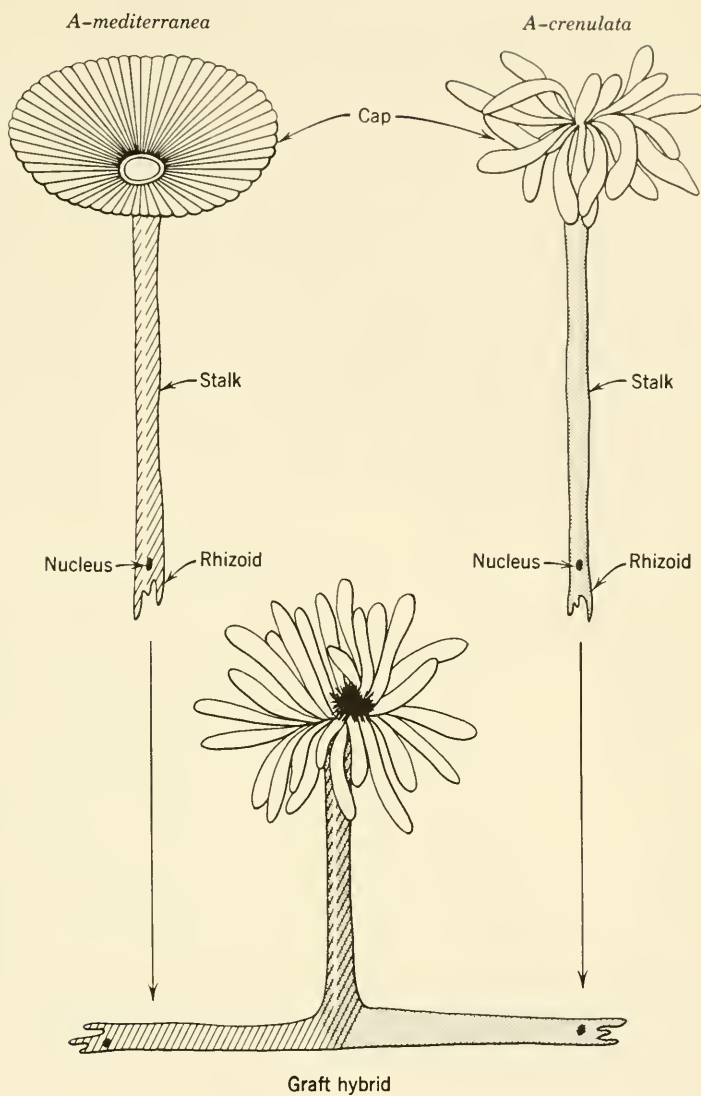


FIGURE 11.1. Graft hybrids of *Acetabularia*. In grafts between rhizoids of the two species, *A. mediterranea* and *A. crenulata*, in which each contributes a nucleus to the hybrid, the morphology of the cap formed after grafting is intermediate, showing an influence from both species. When anucleate segments of the stalk of one species are grafted onto the nucleated rhizoid of the other species, the first cap formed may be intermediate, but subsequently all caps have the morphology of the species which contributed the nucleus (adapted from Hammerling, 1953, *Intern. Review Cytol.*, 2:475).

shall confine our discussion to lines of evidence concerning the control of nucleic acid and protein syntheses.

PROTEIN SYNTHESIS

A major pathway of protein synthesis has been discovered within the last few years, and is being intensively investigated now in many cell systems, including mammalian liver and pancreas, Ehrlich ascites tumor cells, pea seedlings, yeast, and bacteria. This pathway was initially demonstrated by means of *in vitro* incorporation of amino acids into protein in mammalian cell systems by Zamecnik and collaborators, and subsequently confirmed by a number of other investigators. It must be borne in mind, however, that in all these systems, the yield of protein is very low, necessitating caution in the interpretation of results.

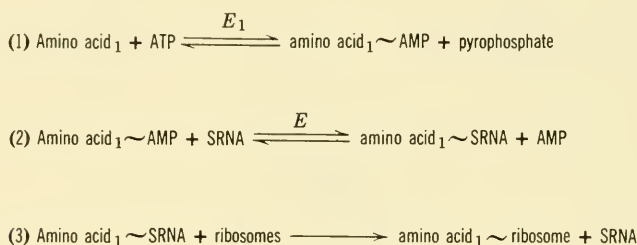


FIGURE 11.2.

As currently understood, and shown in Figure 11.2 this pathway is as follows:

1. Free amino acids are activated by reaction with ATP to form amino acid-adenylate complexes. This reaction is catalyzed by a set of activating enzymes, each one specific for a particular amino acid. These enzymes seem not to be species-specific in activity, since the cell fraction which contains them can be provided from a variety of different cell types, in *in vitro* experiments.

2. The activated amino acid is transferred from this complex to an RNA molecule. There are specific RNA's for each of the amino acids; together they are referred to as the soluble-RNA (*s*-RNA) or transfer-RNA fraction. In *E. coli*, these RNA molecules have an average molecular weight of about 25,000, thus containing some 100 nucleotides. The base ratios so far reported indicate the same kind of complementarity as in DNA, with the molar per cent of adenine equal to that of uracil, and

cytosine equal to guanine. In addition, a number of unusual bases, such as pseudouracil, thymine, 2-methyladenine, 6-methylaminopurine, and 1-methylguanine have been found in this fraction. The terminal nucleotide sequence has been reported to be the same in each molecule, consisting of amino acid-adenylic acid-cytidylic acid-cytidylic acid-RNA. If this is correct, the specificity must reside interior to this sequence. It now seems probable that the same enzyme catalyzes both steps 1 and 2.

3. The activated amino acids are transferred from the *s*-RNA fraction to the ribosomes, which are the site of actual protein synthesis in the sense of polymerization of the polypeptide chain. Subsequently, the proteins are released from the particles into the cell sap. The intermediate reactions between amino acid-*s*-RNA complex formation and appearance of a new protein have not yet been resolved.

In recent kinetic studies with rapidly growing intact cells of *E. coli*, it was demonstrated, by following the incorporation of S^{35} into protein, that the entire process of protein synthesis could occur in a time interval of less than 5 seconds (Figure 11.3). Furthermore, at least 50 per cent of the total cellular protein synthesis proceeded through the ribosomes.

Comparisons between mammalian and bacterial systems are particularly interesting because of the apparent differences between them in cytoplasmic organization (see Chapters 7 and 9). Bacteria appear to have no nucleolus, no nuclear envelope, and no respiratory ATP-

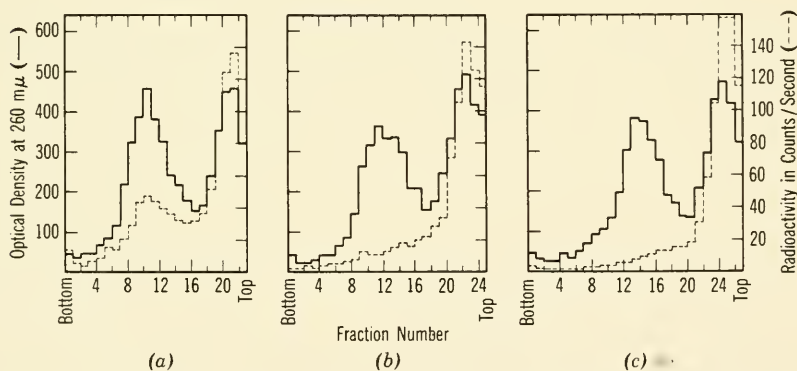


FIGURE 11.3. Flow chart showing the speed of protein synthesis. Intact bacteria (*E. coli*) were treated with radioactive sulphate (S^{35}) then frozen, broken and sedimented in an ultracentrifuge. Solid lines show distribution of RNA fractions; the peak between 8 to 16 on the abscissa represents ribosomes, and the peak from 20 to the top of the cell is S-RNA (a) Cells incubated 15 seconds with $S^{35}O_4^{2-}$. (b) Same as (a) followed by 15 seconds incubation with S^{32} "chaser". (c) Same as (a) followed by 120 seconds incubation with S^{32} "chaser". Note transfer of radioactivity from ribosomes to non-sedimentary region (from McQuillen, Roberts, and Britten, *Proc. Natl. Acad. Sci. Wash.*, 1959, 45:1437).

generating particles like the mitochondria of higher forms; the cytochromes of bacteria are confined to the plasma membrane. Bacteria do have ribosomes, the RNA-containing particles of the cytoplasm, which seem to be comparable with those of mammalian cells. In rapidly growing cells of *E. coli*, the ribosomes contain over 80 per cent of the total RNA.

In mammalian cells, as in bacteria, the bulk of protein synthesis appears to occur on the ribosomes, but the quantitative evidence is less complete than in the *E. coli* system. So far as the analysis of protein synthesis has progressed, no significant differences have been found among plant, animal, and microbial systems.

Although the knowledge about this pathway of protein synthesis is still fragmentary, several points of importance have already emerged. From the viewpoint of the energetics of the problem, the formation of the amino acid-adenylate complex (Figure 11.2) provides the energy for peptide-bond formation, so that, subsequently, when the amino acids are lined up on the protein-forming template, polymerization may be accomplished with little additional energy requirement.

With respect to arrangement of amino acids in the unique array required for protein specificity, it now appears likely that this occurs in or on the ribosomes. Reasoning by analogy with the viruses in which the protein serves as a genetically inert wrapper for the genetically potent nucleic acid, many investigators now view the ribosomal RNA and not the protein as the carrier of the genetic code which determines amino acid sequence and, thereby, protein specificity. One obstacle in this line of thinking had been the difficulty of providing a sterically acceptable model in which a linear sequence of nucleotides could determine a corresponding sequence of amino acids. This particular dilemma has been overcome by the unexpected finding of *s*-RNA as an intermediate carrier of amino acids. On one proposed model, the sequential arrangement of amino acids is determined by base pairing between the ribosomal RNA, which carries the code, and *s*-RNA which carries the amino acids. Among other questions, this leaves unanswered why the *s*-RNA molecules are so large, since on formal grounds it has been shown that no more than three or four nucleotides would be enough to distinguish among 20 different possibilities, i.e., 20 different *s*-RNA's.

One way of studying this problem would be to determine whether the *s*-RNA carries any further specificity beyond that required to discriminate between individual amino acids. If a system for the *in vitro* synthesis of a single well-characterized protein could be established, then one might test the effects of *s*-RNA fractions as well as ribosomal fractions from different sources upon the specificity of the product pro-

tein. Systems of this sort are already being developed for the *in vitro* synthesis of hemoglobin, of tryptophan synthetase, and of β -galactosidase.

In addition to the soluble proteins of the cell sap and the ribosomal proteins themselves, evidence has been found of protein synthesis in the nucleus.

Protein synthesis in the nucleus has been studied in cells of the thymus gland—thymocytes—in which the nucleus constitutes 65 per cent of the dry weight of the cell. Whether the synthetic processes found in these specialized cells also occur in other cell types has not yet been established. In the thymocytes, protein synthesis has been studied both with intact cells and with isolated nuclei, by following the incorporation of radioactive amino acids into protein. Amino acid-activating enzymes and *s*-RNA have been implicated in the pathway, which appears similar to that of cytoplasmic protein. A remarkable distinction of the nuclear system is its reported insensitivity to ribonuclease, an enzyme which instantaneously stops ribosomal protein synthesis by degrading *s*-RNA.

Very recent *in vitro* studies of protein synthesis have demonstrated that the incorporation of C^{14} labeled amino acids into protein requires the presence of ribosomes, of *s*-RNA, and of the amino acid activating enzymes plus an energy-generating system to make the amino acid-*s*-RNA complexes, as well as unidentified enzymes and cofactors present in the supernatant fraction from which the ribosomes have been sedimented. Spectacular results have been achieved with synthetic polynucleotides, such as poly-uridylic acid, which increase the incorporation rates several thousand fold. The added polynucleotides act as templates for protein synthesis, and provide a direct means for examining the coding relations between nucleic acids and proteins. (See Postscript.)

NUCLEIC ACID SYNTHESIS

Our current understanding of the mechanism of DNA synthesis comes primarily from the studies by Kornberg and co-workers of the *in vitro* synthesis of DNA by an enzyme preparation from *E. coli*. As discussed in Chapter 7, the *in vitro* system requires the presence of all four of the deoxyriboside triphosphates and of a pre-existing DNA "primer," as well as the enzyme. The newly formed DNA exhibits the base ratios of the primer, indicating that the primer and not the enzyme determines the composition of the new polynucleotide. With the recent synthesis of a polynucleotide containing only deoxyadenylate and thymidylate, it has been possible to test the primer hypothesis critically. Using the A-T copolymer as primer, the newly synthesized material was found to contain only the adenine- and thymine-containing nucleotides.

The best primer described to date for this system is a single-stranded DNA obtained from the bacterial virus ϕ X174. When the more usual double-stranded DNA is used as primer, it must be opened up in some manner, for example, by heating to break hydrogen bonds.

The localization of DNA synthesis on chromosomes has been demonstrated most directly by autoradiographs of cells after treatment with tritiated thymidine. Because of its very weak β -emission upon disintegration, tritium (H^3) provides the best material for localization of the radioactive atoms within cells at the magnification level of the light microscope. With the use of tritiated thymidine, in a wide variety of cell types, the chromosomes have been described as the unique site of DNA synthesis.

Evidence has been obtained in *E. coli* for the existence of a regulatory mechanism which controls the time of DNA synthesis. Using a double mutant which requires thymine—a constituent of DNA—and histidine—a constituent of protein—it has been shown that each cycle of DNA replication must be preceded or accompanied by protein synthesis.

These observations are particularly interesting in relation to a phenomenon which has been called "thymineless death," and which consists of the exponential death of thymineless cells when incubated in a medium supplying everything necessary for growth except thymine. The cells behave as if the attempt to make DNA in the absence of thymine were lethal. However, cells can be rescued from thymineless death by inhibiting protein synthesis. The regulatory mechanism which blocks DNA synthesis in the absence of protein synthesis also blocks the occurrence of thymineless death.

Studies of RNA synthesis have not yet provided much definitive information. This class of macromolecules, which are of key importance in protein synthesis and, perhaps, as carriers of primary genetic information, have thus far eluded the systematic analysis of their synthesis. The fragmentary evidence available must be considered preliminary.

An enzyme, polynucleotide phosphorylase, which catalyzes the polymerization of ribonucleotides, was discovered by Grunberg-Manago and Ochoa. It has been isolated from bacteria and other cell types. Substrates of the reaction are the diphosphates of the four ribonucleotides found in RNA. If all four nucleotide diphosphates are present, the product will contain them all, but the presence of all four is not obligatory for polyribonucleotide synthesis. No primer need be added to this system, but since the enzyme has not yet been fully purified, it is possible that some unidentified constituent of the enzyme preparation is required for the reaction.

By providing only one nucleotide diphosphate as substrate, it has been

possible to obtain separately, in pure form, polyadenylic acid and polyuridylic acid, both of which have been extensively studied, and a number of other synthetic products as well. These polymers have been extremely valuable in physical chemical studies. One question of importance from the biological point of view concerns the properties and stability of single-stranded nucleic acids. With the synthetic polymers it has been shown that they form hydrogen-bonded double-stranded helices similar to those of DNA, with the bases mainly lying in the inside and the sugar-phosphate backbone on the outside of the helical molecule. Furthermore, three-stranded configurations have been observed, and have provided the basis for speculation that RNA may be synthesized in the cell as a single-stranded polymer in contact with the double-stranded DNA.

The fact that cellular RNA seems only to be single-stranded is an important finding in view of the *in vitro* demonstration that the single-stranded molecules are very unstable in their physical configuration. Their single-strandedness might be an evidence of their need for a specific binding site for activation, or even that different binding sites might make possible different activities.

It is not established just what is the cellular role of polynucleotide phosphorylase, but there is little known as yet of any other RNA-synthesizing enzymes. It seems likely that some RNA may be replicated by copying from pre-existing RNA, in view of the genetic role of viral RNA. With the recent discovery of a single-stranded DNA in the bacterial virus, ϕ X174, more information may soon become available concerning possible mechanisms of synthesis and stabilization of single-stranded nucleic acids within cells.

Only recently have most investigators begun to take into account the known heterogeneity of RNA in their experimental design. With the growing awareness that different classes of RNA are found within cells, performing different functions and localized at different sites, it has become evident that studies of total RNA have limited significance for most questions. Consequently, it is only in recent experiments that attempts have been made to distinguish among classes of RNA; these have begun to shed some light on questions of RNA synthesis and function.

Recent studies with *E. coli* have demonstrated that RNA can be synthesized in the absence of protein synthesis, by uncoupling the two systems with chloramphenicol, provided that catalytic amounts of all amino acids are present. However, this RNA synthesized in the presence of chloramphenicol is unstable, perhaps owing to the absence of its companion protein. The requirement of small amounts of all amino acids for RNA synthesis suggests that an amino acid-nucleotide complex may be an intermediate in RNA synthesis as it is in protein synthesis.

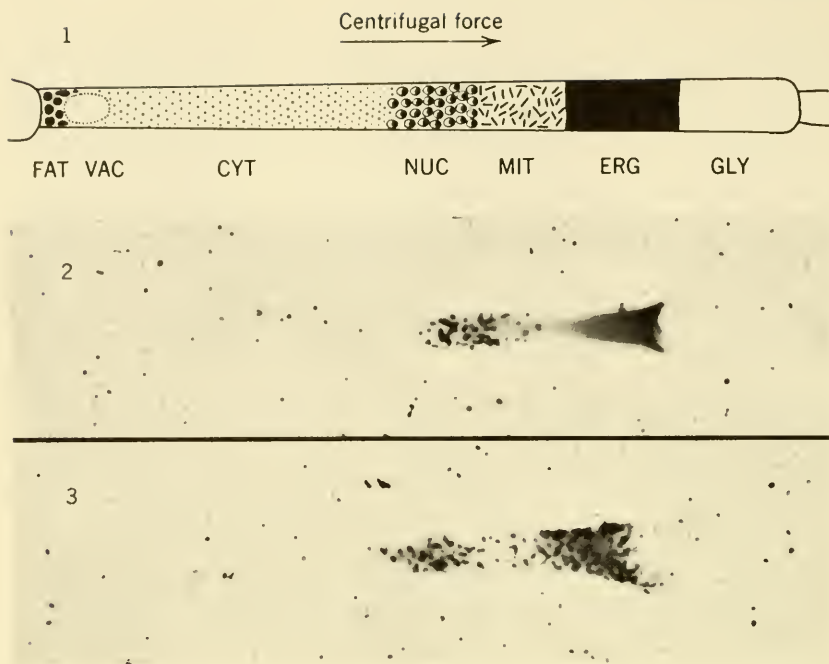


FIGURE 11.4. Centrifuged hyphae of *Neurospora* (after Zalokar, 1960, *Expt. Cell Res.*, 19:559).

1. Schematic diagram showing separation of cellular constituents. FAT, fat; VAC, vacuoles; CYT, enchylema; NUC, nuclei; MIT, mitochondria; ERG, ergastoplasm; GLY, glycogen.

2. Autoradiograph taken after one minute exposure to uridine- H^3 . All detectable radioactivity is associated with the nuclear fraction.

3. Autoradiograph taken after one minute exposure to uridine- H^3 followed by one hour in nonradioactive uridine. Radioactivity is now found associated with the mitochondria and ergastoplasm (endoplasmic reticulum) as well as with the nuclear fraction.

The localization of RNA within the cell has been studied by means of autoradiography, employing P^{32} , C^{14} -nitrogen bases, and, more successfully, tritium-labeled cytidine and uridine with a variety of cell types. Most of these experiments have given similar results, indicating that the bulk, if not all, of RNA synthesis occurs within the nucleus. Two examples of this kind of experimentation will be presented.

A method was developed by Zalokar to centrifuge strands of *Neurospora* mycelium so that the cell constituents are layered within the cell, as if in a centrifuge tube. As shown in Figure 11.4, the microsomes,

mitochondria, nuclei, and cytoplasmic cell sap are sharply delimited from one another. The method lends itself to studies of incorporation patterns after treatments as short as one minute. By feeding mycelia with tritiated uridine for various times, followed by washing and further growth in an excess of nonradioactive uridine, it was shown that, during the first four minutes, radioactivity appears only in the nuclear fraction, subsequently appearing in the cytoplasmic fractions. When the tritiated compound was fed for only one minute, followed by an excess of unlabeled uridine, the radioactivity appeared first in the nuclear fraction and, after several minutes, moved to the cytoplasm. Within the limits of sensitivity of this system, it appears that all the RNA is formed within the nuclei and is subsequently transported to the cytoplasm. The method is ingenious, providing definitive information about the major constituents, but cannot be employed to study constituents present in low amount.

The uptake of radioactive precursors into RNA has also been followed by radioautography of a number of different types of cells. An example of the results are those obtained by Taylor with rapidly growing cells of the Chinese hamster in tissue culture. Using tritiated cytidine as the source of the radioactive label, it was seen that, after exposure of cells for 5 to 10 minutes, only the nuclear RNA contained detectable label. If these cells were washed after their short exposure to cytidine- H^3 , and kept for varying lengths of time in fresh medium with an excess of unlabeled cytidine and uridine, the radioactivity could be followed as it moved from nucleus to cytoplasm. There was no increase in radioactivity in the nucleus after washing out the labeled precursor, indicating the absence of a precursor pool in these cells. Consequently, it was possible to ascribe all changes occurring after washing to movement of labeled material within the cell. As can be seen in Figure 11.5, the disappearance of radioactivity from the nucleus coincided with its appearance in the cytoplasm.

The increase in total radioactivity in the cytoplasm above that initially present in the nucleus has been interpreted by Taylor as the result of more efficient detection of disintegrations in the thin, spread out cytoplasm than in the thick nuclear region. In the absence of an estimate of the quantitative effect of this geometrical problem, the results do not exclude the possibility of some cytoplasmic RNA synthesis, in addition to the transfer of nuclear RNA to the cytoplasm.

Another point of considerable interest in this work was the demonstration of complete cessation of RNA synthesis (as measured by incorporation of cytidine) during the period from prophase to telophase when the chromosomes were highly condensed. There was no apparent decrease

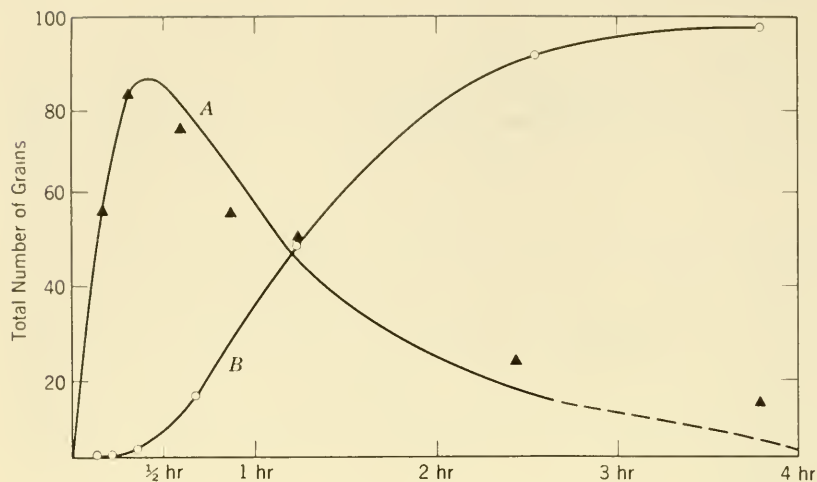


FIGURE 11.5. Incorporation and transfer of RNA in cells of the Chinese hamster in tissue culture. Cells were exposed to cytidine- H^3 for 5 to 10 minutes, then transferred to fresh medium and fixed at the times shown on the graph. Curve A records grain counts of radioautographs over the nucleus and curve B, over the cytoplasm in the same cells. Initial uptake was entirely into the nucleus, and later labeling of cytoplasmic RNA could be ascribed entirely to transfer from the nucleus. (The high final values in the cytoplasm have been attributed to increased efficiency of counting grains in the thin cytoplasm over that of the compact nucleus.) Since the intracellular concentration of RNA may be assumed to remain constant during the 4-hour period, the plotted points are comparable to relative specific activities (after Taylor, 1960, *J. Biophys. & Biochem. Cytol.*, 7:455).

in the rate of RNA synthesis during the prolonged (six hours out of a 14-hour doubling time) period of DNA synthesis. This observation provides no direct information on the question whether DNA and RNA molecules may be replicated simultaneously from the same template, because DNA synthesis occurs in different parts of the chromosome complement at different times during the six-hour replication period.

These experiments, as well as others of a similar nature, have shown that RNA synthesis occurs primarily, if not exclusively, in the nucleus in a variety of cell types. It is possible that these findings apply only to the particle-bound RNA of high molecular weight, since it has not been fully demonstrated whether the *s*-RNA or other fractions of low molecular weight are retained by the fixation techniques employed.

Having focused our attention upon the nucleus as the site of RNA synthesis, we may ask where in the nucleus does this process occur? Is it associated with the chromosomes, the nucleolus, or both? Attempts by

cytologists to answer this question have been severely limited by the invisibility of the chromosomes, except during mitosis and meiosis, under the light microscope. As yet only the polytene chromosomes in certain tissues of some insect larvae, which remain visible throughout larval development, have provided material for a detailed analysis of intranuclear syntheses of DNA, RNA, and protein along the chromosomes in interphase. These chromosomes are magnificent with respect to size, morphological detail, correlation with genetic analysis (in *Drosophila*), and accessibility for short-term labeling experiments. Their principal shortcoming is that they occur in larval tissues which degenerate with the onset of pupation. The close correlation of salivary gland chromosome cytology with genetic events in *Drosophila* has provided a firm basis for viewing the salivary-gland chromosome as a trustworthy mirror, reflecting such changes as chromosomal rearrangements which have occurred. In using these chromosomes to study macromolecular synthesis, however, the investigator makes a new inference, namely, that in late larval development, these chromosomes are still actively integrated in the metabolism of the organism, and reflect its synthetic capacities.

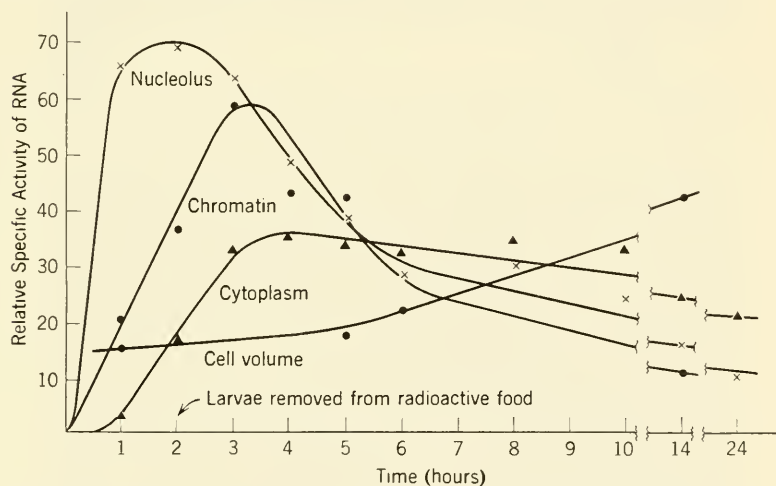
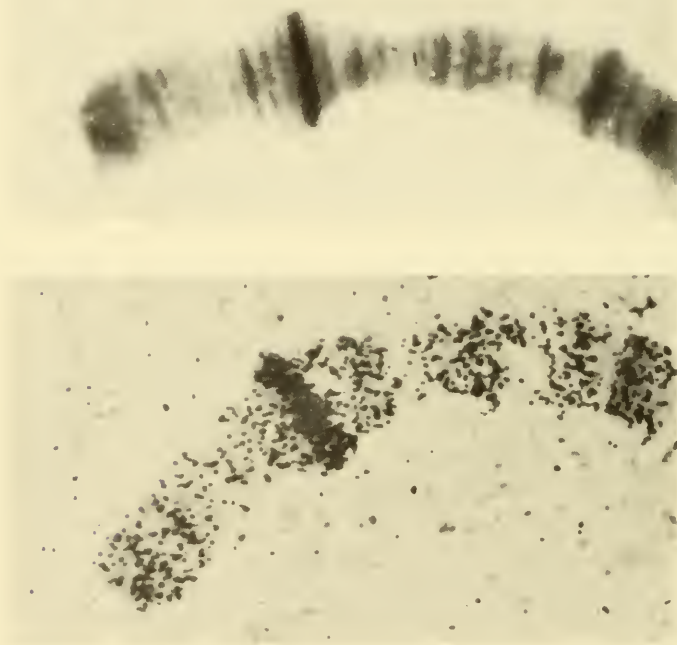


FIGURE 11.6. Incorporation of adenine- C^{14} into the RNA of cell fractions of developing *Drosophila* larvae. Determinations of total RNA by photometric methods and of new RNA synthesis by grain counts of autoradiographs of C^{14} incorporated into RNA, made possible an estimate of relative specific activity of radioactive RNA in various cell fractions in the salivary gland of *Drosophila repleta* larvae. The resulting data demonstrates that uptake into the nucleolus precedes and exceeds that into the chromosomes, which are well separated in this favorable material. The label appears in the cytoplasm considerably later (from Taylor and Woods, 1959, in: Hayashi, *Subcellular Particles*, Ronald Press, p. 172).

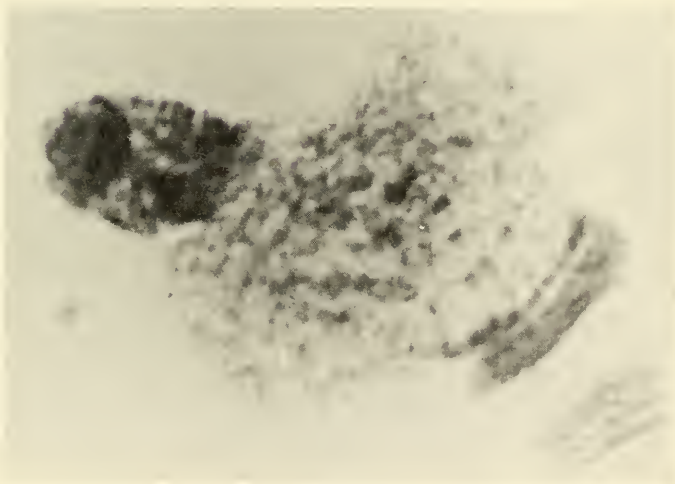
In larvae of *Drosophila repleta*, the cells of the salivary gland grow rapidly during the last few days before pupa formation. During the period of the experiment to be described, the nuclear and cytoplasmic volumes tripled, and the nucleolus doubled its volume and RNA content. Larvae selected for uniformity were fed for two hours with adenine- C^{14} , and then removed to a nonradioactive food source, prior to fixation at various times. The specific activities of RNA in nucleolus, chromatin, and cytoplasm were determined by grain-counting on autoradiographs of sectioned material. The results indicate (Figure 11.6) that RNA synthesis, as measured by the uptake of adenine, proceeds faster in the nucleolus than in any other fraction and, consequently, that neither chromosomal nor cytoplasmic RNA can be the source of all nucleolar RNA. No other information about transfer of label between chromo-



11-7a

FIGURE 11.7. Distal end of salivary chromosome C of *Rhynchosciara angelae* showing localization of DNA by means of autoradiography of tritiated thymidine incorporated into DNA in comparison with Feulgen staining.

(a). The larva was injected with tritiated thymidine 24 hours before being killed. The close correlation between bands of DNA which stain intensely with Feulgen reagent



11-7b

and regions which incorporate much thymidine is evident (from Ficq and Pavan, unpublished).

(b). The larva was injected with tritiated thymidine seven days before being killed, at a time when puffing occurs in this region. It is evident that tritium (and therefore presumably DNA) is present throughout the puff (from Pavan and Sanaia, unpublished).

somal and nucleolar RNA can be deduced from these data. The fact that there are breaks in both curves after five hours suggests the presence of two different RNA fractions at each site, but they have not been identified further.

A much more detailed picture of the synthetic activities of chromosomes has come from autoradiographic studies of particular sites in the polytene chromosomes of such dipteran insects as *Chironomus*, *Sciara*, and *Rhynchosciara*, in which the larval chromosomes attain even greater magnitudes than those of *Drosophila*. Changes in morphology have been observed in these chromosomes, and referred to as "puffs," and "Balbiani rings." Recently, these structural changes have come under careful study in an attempt to correlate them with functional activities of the chromosomes.

Beerman has studied *Chironomus* chromosomes, comparing the morphology of salivary-gland chromosomes with those of the polytene chromosomes of the Malpighian tubules, the midgut and the rectum. He reports differences in the number and location of Balbiani rings and puffs in different tissues at different times in development. Puffs have been followed carefully through larval development in *Rhynchosciara* by Breuer and Pavan, who found that the process of puffing is entirely reversible, although the timing varies from locus to locus. Altogether, few puffs are found per chromosome compared to the number of bands.

Recently, the uptake of radioactive precursors has been employed to investigate whether macromolecular syntheses occur in relation to puff formation. In a number of different species, it has already been shown that DNA, RNA, and protein synthesis may occur in conjunction with puffing. The three types of compounds may each be synthesized independently of changes in the others, or simultaneous syntheses may occur. As shown by the photograph of Figure 11.7, the autoradiographs of these giant chromosomes are dramatic evidences of high rates of synthetic activity. Is puff formation a visible expression of gene action? This is the working hypothesis of investigators in this field.

To summarize the preceding section, we have seen that protein synthesis occurs primarily in cytoplasmic RNA-protein particles, the ribosomes; that two classes of RNA molecules have been identified with the process, s-RNA which carries the activated amino acids, and another RNA, which is conjectured to provide a template for specific sequential arrangement of the amino acids during polymerization. Protein synthesis also occurs in the nucleus at least in some cell types. The synthesis of most, if not all, RNA occurs in the nucleus; consequently, it is hard to escape the conclusion that ribosomal RNA *per se* is synthesized in the nucleus, despite the indirect nature of the evidence. An unidentified

protein seems to play a role in initiating each cycle of DNA synthesis in *E. coli*, and one may speculate that the timing of DNA synthesis is also under precise cellular control in other forms.

A prevalent current hypothesis states that the genetic specifications (information) carried in the DNA are transferred to RNA by DNA-RNA pairing during RNA synthesis, and that this RNA, in turn, is built into ribosomes which function in the cytoplasm as the sites of protein formation. One weakness of this hypothesis is its lack of discrimination among the various classes of RNA and perhaps even of DNA, as well as of protein. As with the categories of "nuclear" and "cytoplasmic," those of DNA, RNA, and protein are too sweeping.

A great refinement in methodology has been contributed by investigations of the formation of individual, identified proteins, coupled with an analysis of the genetic determinants which control their specificity and rate of formation. As we shall see, studies of the metabolic control of enzyme formation have led to some of the most direct findings concerning the intimate involvement of genes in this process.

GENETIC CONTROL OF ENZYME FORMATION

It is not a rare occurrence in research that a problem appearing relevant within one frame of reference early in its study develops along unpredicted lines and later becomes centrally important in quite a different context. The study of enzyme adaptation in microorganisms has had many unexpected turns in its history, and seems now to be reaching a focal point of development, uniting many divergent lines of research and providing the basis for a more penetrating analysis of cell integration.

The phenomenon of enzyme adaptation, that is, the change in enzyme content of cells occasioned by specific environmental changes, was first described in microorganisms before 1900. It was shown, for example, that yeast cells grown on glucose do not contain the galactose-fermenting enzyme, galactozymase, but that after a delay (lag period), in the presence of galactose, such cells will begin to ferment that sugar, gradually increasing their galactozymase activity. This kind of phenomenon was observed widely among microorganisms, and aroused interest in terms of the economy of the cell, which seemed to be manufacturing enzymes only when needed, and in terms of possible mechanisms by which this environmental control might be elicited. After a long quiescent period, interest in enzyme adaptation reappeared in the mid-1930's, and has been accelerating ever since. At present, this field is providing some of the best material for the integrated analysis of protein synthesis,

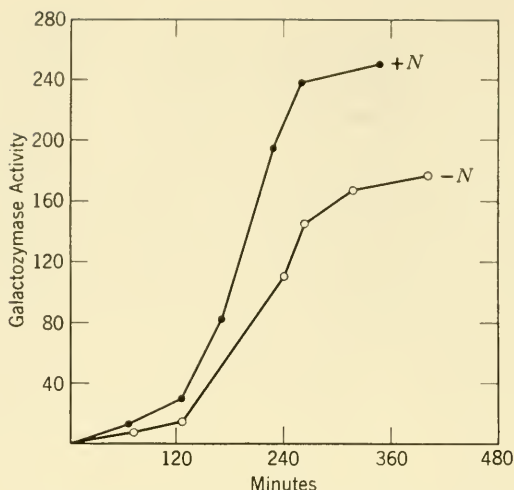


FIGURE 11.8. Induction of galactozymase in yeast cells in the presence and absence of a nitrogen source. Yeast cells grown on glucose have no galactozymase activity, but following the addition of galactose, enzyme formation begins. Within 4 hours, considerable enzyme is formed both in the presence and absence of a nitrogen source. These results indicate that a new enzymatic activity can appear in nondividing or scarcely dividing cells (after Spiegelman, 1946, *C. S. H. Symp. Quant. Biol.*, 11:256).

gene action, and regulatory mechanisms of metabolic control at the cellular level. In what follows, we shall attempt neither a chronological development of the subject nor a comprehensive summary of the field, but rather a sketch of those findings and related concepts which appear to be central to the analysis of mechanisms of genetic control.

The most extensive, systematic, and diversified analysis of enzyme formation has been performed by Monod and collaborators over a period of many years at the Pasteur Institute, focusing primarily upon the β -galactosidase enzyme of *E. coli*. Other contributions of central importance have come from Spiegelman's studies with yeast, and Pollock's investigations of penicillinase formation in the bacterium *Bacillus cereus*.

Consider a population of yeast cells growing in liquid culture with glucose as their carbon source. They are washed, and half of them are resuspended in a growth medium containing galactose as sole carbon source, the other half in a medium with galactose but no nitrogen source to prevent growth. The resulting increase in activity of the galactose-fermenting enzyme, galactozymase, is shown in Figure 11.8. Almost as

much enzyme is produced in nondividing cells as in growing cells within a short time interval. From the sigmoid shape of these curves, one might suppose that they reflect a population heterogeneity—that initially only a small fraction of the cells could utilize galactose, and that the curve really represents the increase of that fraction in the population, as it becomes the predominant cell type. This possibility is effectively ruled out by the similarity of the curve found in the absence of a nitrogen source. It is seen that a new enzymatic activity can arise in scarcely dividing yeast cells, which also are not dying, as shown by plating at intervals to determine their viability. In this experiment, the appearance of the enzyme galactozymase has been *induced* by galactose, which is termed the *inducer*.

Similar results have been obtained with a number of enzyme systems in yeast and in bacteria. With more sophisticated techniques, it has been possible to demonstrate in two systems (β -galactosidase formation and penicillinase formation) that virtually every cell in the population behaves uniformly in altering its enzyme content in response to the new environment. *Thus, enzyme adaptation in these systems is a cellular phenomenon, not a population shift.*

Further evidence in support of this conclusion comes from studies of the deadaptation process, in which the enzyme content per cell decreases in the absence of an inducer, at a rate far greater than could be attributed to differential growth rates of a heterogeneous population of cells.

To analyze the system further, it is important to investigate the origin of the enzyme itself. Does it appear by some subtle change in a pre-formed protein, or is it synthesized *de novo* from its constituent amino acids? This question has been decisively answered with the enzyme β -galactosidase of *E. coli*, and galactozymase of yeast, employing a number of different methods. In one type of experiment, cells were grown on glucose in a medium containing S^{35} until all proteins were effectively radioactive. Then the cells were washed and resuspended in a nonradioactive medium containing an inducer of the enzyme. After appearance of enzyme activity, β -galactosidase was extracted from the cells and purified. As purification proceeded, the radioactivity of the preparation decreased, until finally the highly purified enzyme, characterized both enzymatically and chemically, was virtually free of all S^{35} . This experiment is a stringent test of the source of the sulfur-containing amino acids of the enzyme, showing definitely that they come from the nonradioactive amino acid precursors in the medium at the time of induction, and not from pre-existing proteins within the heavily radioactive cells. It may therefore be concluded that in this system at least,

TABLE 11.1

Inducers of β -Galactosidase(From Monod and Cohn, 1952, *Advances in Enzymol.*, 13:102)

	1	2	3
1. Galactose	420	30	0
2. Methyl- β -D-galactoside	2800	10	+
3. Phenyl- β -D-galactoside	560	600	+
4. o-nitrophenyl- β -D-galactoside	1060	1000	+
5. 4-glucose- β -D-galactoside (lactose)	2500	100	+
6. Phenyl- β -D-thiogalactoside	0	700	-
7. Methyl- α -D-galactoside	140	0	-
8. 6-glucose- α -D-galactoside (melibiose)	2400	0	-
9. L-arabinose	0	>0	-

Column 1: Activity induced by $10^{-3}M$ inducer.

Column 2: Relative affinity for the purified enzyme.

Column 3: Hydrolysis by the purified enzyme.

enzyme adaptation involves the synthesis of new protein from constituent amino acids.

It must be noted, however, that the occurrence of enzyme synthesis has been shown in only a few of the many systems involving enzyme adaptation. The appearance of a new protein should be demonstrated by chemical as well as enzymatic criteria.

The nature of the inducer has been studied in a number of systems, the data being most extensive for β -galactosidase of *E. coli*. The inducing ability of various compounds has been compared with their capacity to serve as substrates of the enzyme, by measuring the relative rates of induced enzyme formation in comparison with growth studies employing the inducer as sole carbon source. Some results are shown in Table 11.1.

It can be seen that many kinds of galactosides can serve as inducers and as substrates. However, *not all substrates are inducers, and not all inducers are substrates*. This is a very important finding, for it indicates that, although there is a close similarity, the active site of the enzyme is not identical with the postulated site of action of the inducer.

In consequence of these findings, the phenomenon of enzyme adaptation has been redubbed more pointedly, "induced enzyme synthesis." An example of the inducer-substrate relation is provided by comparing two galactosides with their thiogalactoside analogues, as shown in Figure 11.9. Neither of the thiogalactosides can be hydrolyzed by the enzyme, but the methyl derivative (TMG) is a potent inducer. The inducing ability of TMG is shown dramatically by the growth of cells on a com-

bination of TMG and phenyl galactoside, neither of which will support growth alone.

Comparative studies of the binding affinities for different galactosides and of the immunological specificity of purified preparations of β -galactosidase, produced after induction by various inducers, have revealed no differences among them. Within the sensitivity limits of the methods employed, these results show that the inducer does not confer any specificity upon the induced enzyme. Since it is usually possible to detect a

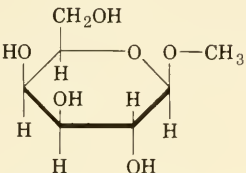
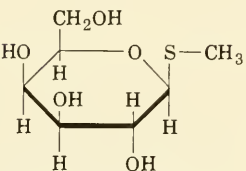
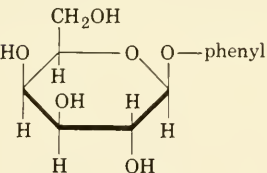
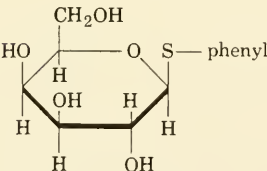
	Inducer	Substrate
 Methyl-β-D-galactoside	+	+
 Methyl-β-D-thiogalactoside	+	-
 Phenyl-β-D-galactoside	-	+
 Phenyl-β-D-thiogalactoside	-	-

FIGURE 11.9. Galactosides and thiogalactosides as inducers of β -galactosidase and as substrates of that enzyme in *E. coli* (from Monod, 1956, *Enzymes: Units of Biological Structure and Function*, New York, Academic Press, pp. 7-28).

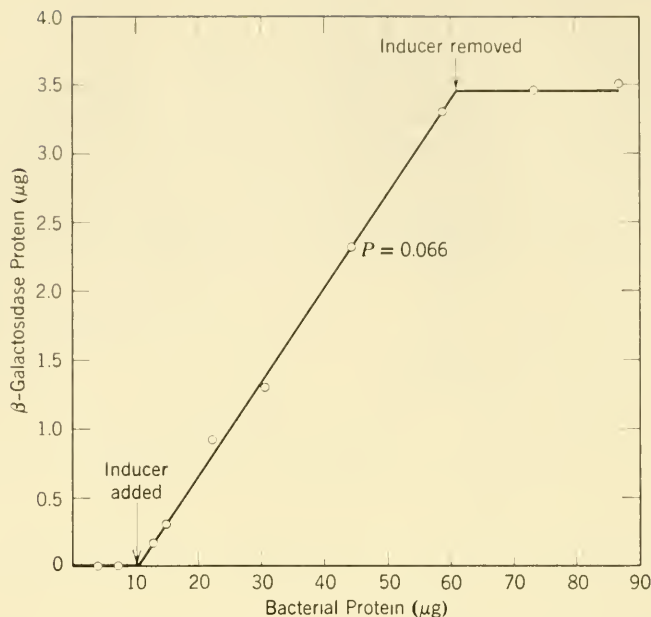


FIGURE 11.10. The differential rate of synthesis of an induced enzyme. The kinetics of formation of β -galactosidase in *E. coli* are revealed by plotting enzyme activity against total bacterial protein over a suitable time period. When inducer is added, enzyme formation begins before the first measurement is made, and continues at a rate which is a constant fraction of total protein synthesis in the growing population. When inducer is removed, enzyme synthesis ceases and the molecules present are diluted out among the multiplying cells (from Monod, 1958, *Recueil des travaux chimiques des Pays-Bas*, 77:569).

small amount of basal enzyme present in uninduced cells, induction has been viewed as the increased production over the basal level of an enzyme which the cell already knows how to make.

A related finding of importance has come from studies of induced synthesis of penicillinase in *Bacillus cereus*. The number of bound penicillin molecules which serve as inducers of penicillinase has been estimated by the use of radioactive penicillin, to be in the range of 100 molecules per cell or less. Since penicillinase induction leads to the presence of some 25,000 molecules per cell, it is evident that the role of the inducer is a catalytic one with respect to enzyme formation.

It now becomes relevant to look more directly into the process of enzyme formation itself, by examining its kinetics. The curves in Figure 11.8 show a greater than linear increase in enzyme activity over time,

but attempts to analyze these curves are hopelessly complicated by the changing metabolic state of the cells. Ideally, the kinetics of enzyme formation should be studied against a background of homogeneity, both of cell type and metabolic state, in every respect other than formation of the new enzyme. Furthermore, since the internal concentration of inducer may be very important, it would be useful to employ an inducer which is not metabolized. This ideal state has been approximated in the β -galactosidase system by the use of TMG as inducer, with rapidly growing young cultures. Under these circumstances, the differential rate of formation of the enzyme is constant over time, from the moment of induction, as can be seen in Figure 11.10. The increase in enzyme is plotted against the increase in total protein; the resulting linearity demonstrates that the amount of enzyme being synthesized is a constant fraction of the total cell protein being formed. It is under conditions in which the differential rate of enzyme synthesis remains constant, or is altered in a controlled manner, that the process of enzyme formation can best be analyzed.

It is also useful in some studies to plot the data in the form of enzyme content per cell against time when the effects of environmental changes are being investigated. Plotted in this way, at a constant differential rate of synthesis, enzyme concentration per cell will approach a constant maximum value in about four generations after induction. Studies of arginine synthesis under conditions which violated these kinetics provided some of the first evidence that the control of enzyme formation involved more parameters than just the presence or absence of an inducer in an otherwise competent cell.

Let us consider the pathway of arginine biosynthesis, shown in Figure 11.11. Several of the enzymes of this pathway have been studied; here we shall discuss ornithine *trans*-carbamylase (OTC-ase). When wild-type cells of *E. coli*, strain W, are grown on a minimal medium, the

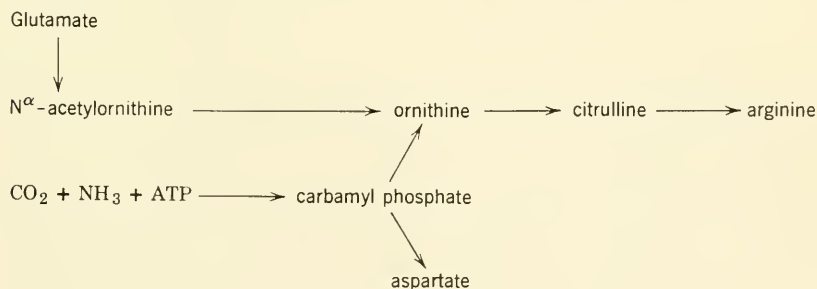


FIGURE 11.11. Pathway of arginine biosynthesis in *E. coli*.

concentration of this enzyme and the rate of its formation have constant values which we shall consider the norm. When these cells are grown with arginine supplied from the medium, formation of the enzyme stops abruptly and its concentration falls by dilution during subsequent growth. When those arginine-grown cells are washed and resuspended in minimal medium, they begin to synthesize OTC-ase again, as if it were being induced. The phenomenon resembles induction in reverse: the presence of arginine inhibits enzyme formation, and the absence of arginine induces it.

These observations have a number of interesting aspects; let us begin with the role of arginine. The effect of arginine in the medium upon the content of OTC-ase per cell is shown in curves *B* and *C* of Figure 11.12. Cells grown with arginine contain little or no enzyme, and cells grown without arginine level off at about two units per cell, after an unexpected overshoot. The expected kinetics for induction at a constant differential rate are shown in curve *A*. Analysis of the overshoot indicated that it resulted from a temporary very low level of endogenous arginine within the cells. It was shown that withdrawal of arginine from the medium led to the rapid appearance of all the enzymes of the arginine biosynthetic chain, which began to make arginine. As long as very little arginine accumulated within the cells, enzyme synthesis continued, and soon excess endogenous arginine was being formed. It was postulated that this excess served to repress further enzyme formation, and that by the third cell division the system had equilibrated. The most direct evidence that endogenous arginine itself (or a derivative) has a controlling role in synthesis of the enzymes of the arginine pathway comes from studies with the chemostat. Using an arginine-requiring mutant, blocked before ornithine and capable of making the enzyme OTC-ase, it was shown that the content of enzyme per cell under equilibrium conditions was a function of the intracellular arginine concentration (as set by the growth rate of the cells on limiting arginine). At its slowest growth rate, the mutant contained 25 to 50 times the enzyme concentration of the wild-type cells grown without arginine. At various intermediate growth rates, corresponding enzyme concentrations were found. As a control, wild-type conditions could be simulated by using a histidine-requiring mutant growing on limiting histidine in the chemostat. These cells were found to contain the same OTC-ase concentration as do wild-type cells.

It is thus evident that enzyme formation can be repressed as well as induced. Furthermore, the cell is able to regulate enzyme formation with great sensitivity within the wide range between zero and maximal rate. Similar lines of evidence of enzyme repression have been established with a number of different systems. In the biosynthetic pathways

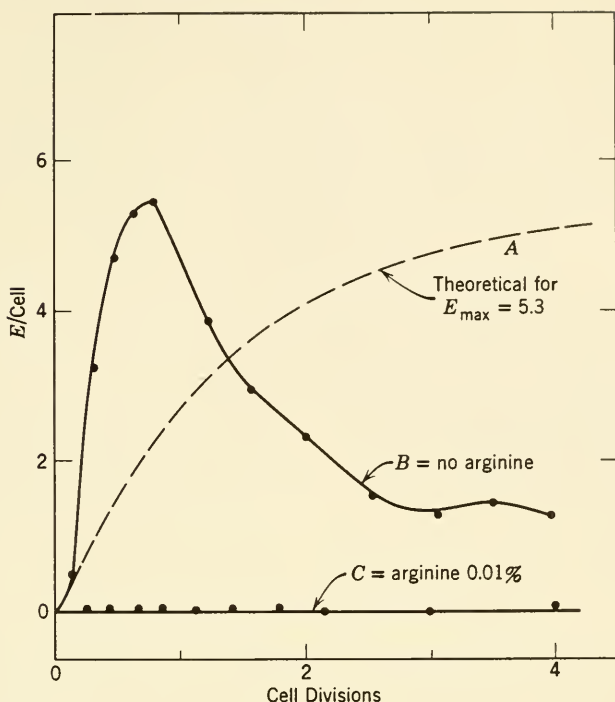


FIGURE 11.12. The role of arginine in controlling the rate of synthesis of ornithine transcarbamylase in wild type cells of *E. coli*. Enzyme activity per cell is plotted against time in cell divisions to demonstrate rapid fluctuations in response to arginine. Curve A is based upon induction kinetics of the type shown in Figure 11.10. Curve C shows the total absence of enzyme activity in the presence of arginine, which is a repressor of ornithine transcarbamylase formation. In the absence of arginine (curve B), enzyme activity levels off at about 2 units per cell, after a sudden initial rise to more than 5 units per cell. The overshoot results from a temporary absence of intracellular arginine, soon followed by establishment of a pool which regulates the enzyme activity at about 2 units per cell (from Gorini and Maas, 1958, in: McElroy and Glass, (eds.), *Chemical Basis of Development*, Baltimore, Johns Hopkins Press, p. 463).

of several amino acids, of the purines and pyrimidines, the end product can repress the formation of all the enzymes along the pathway. An extensive analysis of histidine biosynthesis has shown that the relative rates of formation of four of the enzymes of this pathway are closely correlated, and all are controlled by the histidine concentration of the cells, not by the concentrations of any intermediate substrates. An example of these data is shown in Figure 11.13.

Now we may ask: What is the relation between induction and repression? The existence of an intimate connection of some sort can al-

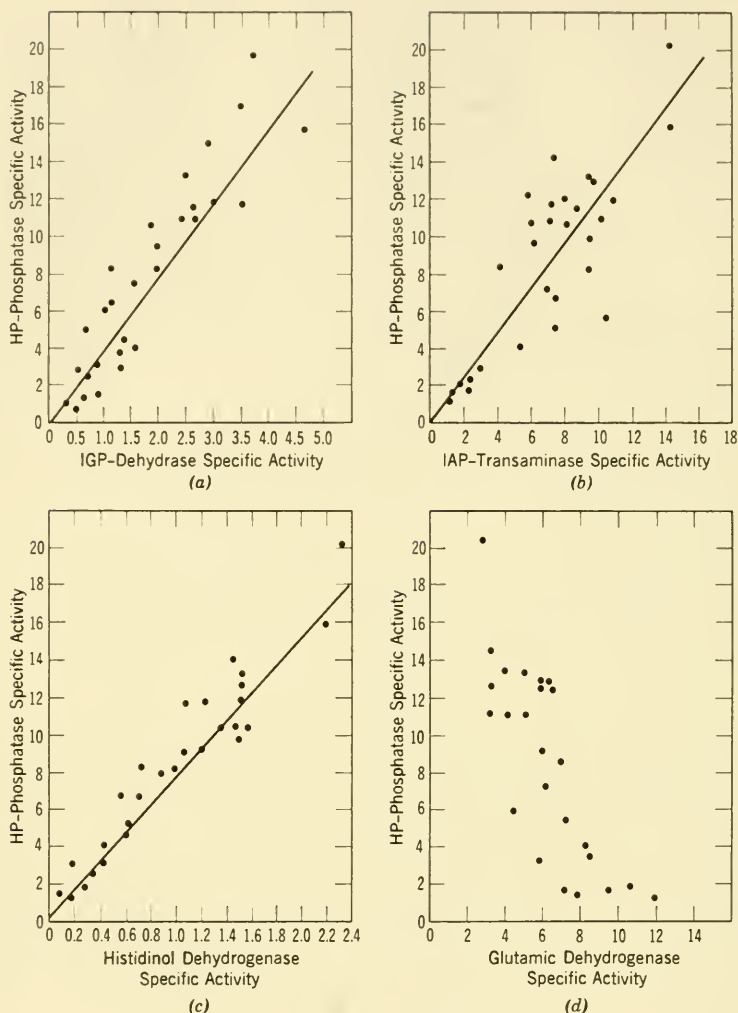


FIGURE 11.13. Coordinate repression by histidine of the formation of enzymes concerned with histidine biosynthesis in *Salmonella*. Four enzymes catalyzing consecutive steps in histidine biosynthesis are (a) imidazoleglycerol phosphate dehydrase; (b) imidazole acetol phosphate transaminase; (c) histidinol phosphate phosphatase; and (d) histidinol dehydrogenase. The enzyme glutamic dehydrogenase is on another pathway and was assayed in these experiments as a control. The specific activity of each enzyme was measured in wild-type and mutant cells grown under various conditions of internal histidine concentration, resulting in a wide range of enzymatic activities per cell. In each instance, the relative activity of all four enzymes of the histidine pathway correlated very closely, indicating a coordinated control of the rates of their formation. This correlation was not observed in the case of glutamic dehydrogenase, which is not directly on the histidine pathway (from Ames and Garry, 1959, *Proc. Natl. Acad. Sci. Wash.*, 45:1453).

ready be glimpsed from the existing data, although the identity of the actual operating inducer and repressor molecules is unknown. They have been pictured as structurally related and competing metabolites. Both inducers and repressors show some structural resemblance to the substrates of the enzyme whose synthesis they regulate, but they are not necessarily identical with it. This evidence has led to the suggestion that inducers and repressors may combine with the enzyme, not at its "active site" but at a related "controlling site."

Although the study of repression of enzyme synthesis is very recent, some models have already been developed. For instance, it is proposed that repressors block enzyme formation by tying down the newly formed protein molecules onto the site of their synthesis. The repressor is visualized as a bipartite molecule with a metabolite-moiety, which combines with the controlling site of the enzyme, and an *R*-moiety, which may be a polynucleotide, capable of combining with a nucleotide sequence of the enzyme-forming site. The inducer is an analogue of the metabolite-moiety, and can compete for the controlling site. When the inducer wins, the enzyme is liberated, and enzyme formation can proceed. Thus, the rate of enzyme formation can be delicately controlled by the ratio of inducer-to-repressor molecules.

This model provides a number of potentially rate-limiting reactions from different metabolic pathways, any one of which could set the final rate of enzyme formation. They include synthesis of inducer and repressor molecules, and of a condensing enzyme. The bipartite repressor is of particular interest, since the *R*-moiety may be a primary gene product, representing a direct pipeline of gene control, while the metabolite-moiety as well as the inducer may speak for the state of the cell milieu.

Repression has been observed not only with the enzymes of biosynthetic pathways but also in the formation of other enzymes. In the β -galactosidase system, the analysis has been complicated by the changing permeability of cells to the inducers, repressors, and substrates being investigated. To clarify what follows, we must digress briefly to describe some recent developments in the understanding of cell permeability.

Permeases

When wild-type cells of *E. coli* are grown on a carbon source such as succinate, their differential rate of synthesis of β -galactosidase after adding an inducer, such as TMG (10^{-4} M), is constant. However, as can be seen in Figure 11.14, at low concentrations of inducer, these simple kinetics are no longer obtained. An understanding of this phe-

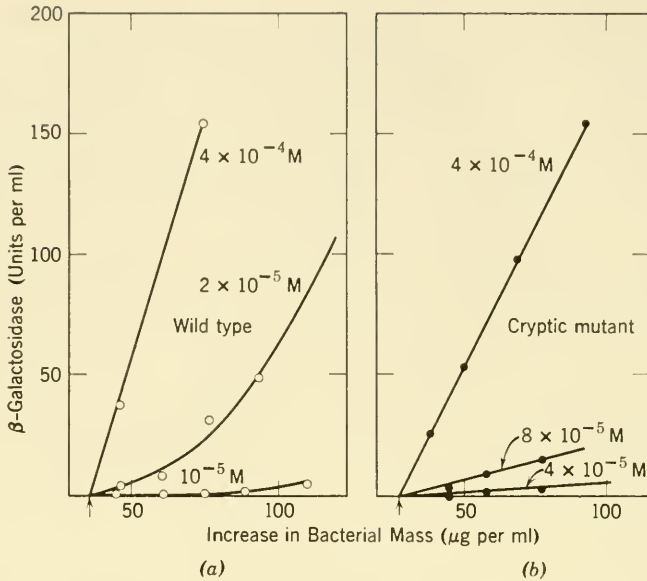


FIGURE 11.14. Comparative kinetics of β -galactosidase formation by wild-type and cryptic mutant cells of *E. coli* in response to induction by methyl- β -D-thiogalactoside. Wild type cells exhibit a constant differential rate of enzyme formation at high but not at low inducer concentrations. The cryptic mutant shows a constant differential rate at all effective inducer concentrations. The difference in response of the two strains results from the presence in wild-type cells of a permease which must be induced before enough inducer can enter the cell to initiate β -galactosidase formation. Consequently, the kinetics of both permease and β -galactosidase formation are reflected in graph (a), but only the latter enzyme in graph (b), since the cryptic mutant lacks permease (from Cohn, 1958, in: McElroy and Glass, (eds.), *The Chemical Basis of Development*, Baltimore, Johns Hopkins Press, p. 458).

nomenon has involved the demonstration of the role of permeability in induction.

When cells are preinduced by growth in the presence of a galactoside, and then washed and grown in the presence of radioactive TMG-S³⁵, they rapidly accumulate TMG to a concentration more than 100 times that of the surrounding medium. This TMG is held within the cells but can be released, as demonstrated by adding an unlabeled galactoside and following the loss of radioactivity (Figure 11.15). The accumulation apparently involves a stereospecific kind of active transport. Energy is required, and so is the ability of the cell to synthesize protein. A high order of stereospecificity is required both for ac-

cumulation and for its reversal. When the intracellular accumulation of TMG is plotted against bacterial growth, a linear relationship is found, similar to that observed in induced enzyme formation (Figure 11.15). From these and related observations, it has been inferred that the entrance of galactosides into *E. coli* requires the induced formation of a "permease," which is considered on indirect evidence to be a protein. This galactoside-permease is referred to in the literature as Y.

In this system, the autocatalytic curves found at low inducer concentrations (Figure 11.14) result from the special properties of permease formation, and do not reflect directly the kinetics of β -galactosidase formation. The most direct demonstration that β -galactosidase kinetics are constant at all inducer levels is provided by studies of a mutant strain which lacks the galactoside-permease.

This mutant, called "cryptic," does not grow on lactose, but when grown, for example, on succinate, will respond to certain galactosides, such as TMG, by forming β -galactosidase in high titer. Cells which are full of the enzyme still do not grow on lactose although, when broken, they hydrolyze lactose very well. Clearly, external lactose does not reach the internal enzyme. With these cells, there should be no effect upon

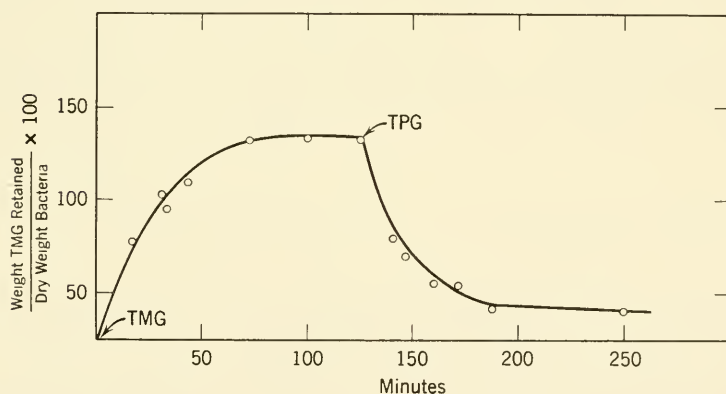


FIGURE 11.15. Uptake and release of inducer showing ability of *E. coli* cells to concentrate TMG intracellularly. The uptake of the inducer methyl- β -D-thiogalactoside- S^{35} (TMG)- S^{35} was measured in induced cells, which may concentrate it up to 4 per cent of their dry weight. Subsequently, addition of the competitive inhibitor phenyl- β -D-thiogalactoside (TPG) displaced the TMG rapidly from the cells. Because of the uptake of high concentrations of inducer, the permease is considered a catalyst rather than a binding site (from Monod, 1956, *Enzymes: Units of Biological Structure and Function*, New York, Academic Press, pp. 7-28).

the differential rate of enzyme formation resulting from the use of low inducer concentrations, and the results in Figure 11.14 bear this out.

Now let us return to the question of repression in the β -galactosidase system. Glucose has been found to block the induced formation of many enzymes of carbohydrate metabolism, and in some systems, glucose acts by repressing permease formation, and thereby keeping potential inducers of internal enzymes out of the cell. The detailed analysis of "the glucose effect" is still in progress, but it already seems likely that its action will, in principle, be found comparable with end-product repression in biosynthetic pathways. Another evidence of similarity between these systems is the fact that permeases have been found which determine uptake of amino acids, as well as of organic acids and other carbohydrates. The repression of a permease involved in the arginine pathway has been reported.

In the past, metabolic control has been studied primarily in terms of substrate availability, on the assumption that the enzymes were not limiting. These recent investigations have shown that the control of *enzyme formation* and, thereby, of enzyme concentration plays a significant regulatory role. The precise interplay between substrate levels and enzyme levels in the over-all control of metabolism has scarcely been explored as yet. From the point of view of genetic control devices, however, it is becoming clear that genes exert a continuous influence upon cell metabolism, and that they operate *via* the control of the rates of enzyme formation. Having considered primarily the effects of inducers and repressors in wild-type cells, let us now turn to comparative studies of mutants to get a picture of the role of the genes in this process.

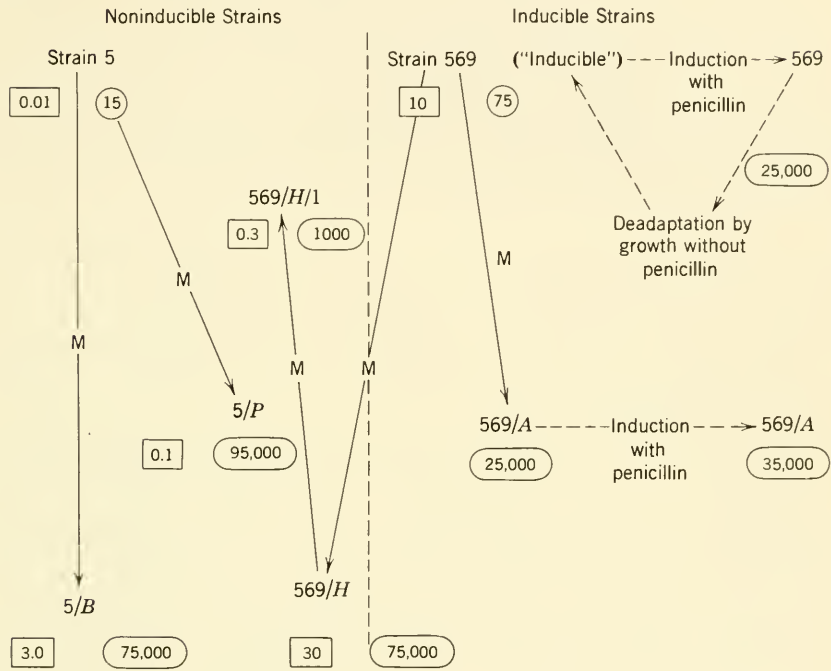
MUTATIONS AFFECTING RATES OF ENZYME FORMATION

Mutational effects upon the penicillinase system are summarized in Table 11.2. An inducible strain with a low basal content of penicillinase, which produces high amounts of the enzyme only when induced, has given rise by mutation to so-called "constitutive" mutants which produce much enzyme (25,000 molecules per cell in one instance, 75,000 in another) in the absence of penicillin. A noninducible strain, with a basal content of only 15 molecules of penicillinase per cell, has given rise to other noninducible strains producing high titers of the enzyme all the time. Thus it appears that inducibility *per se*, as well as basal enzyme level is under genetic control.

In the β -galactosidase system, a number of classes of mutants have

TABLE 11.2
Mutations Affecting Formation of the Enzyme Penicillinase in
Strains of *Bacillus cereus**

(After Pollock, 1959, in: *The Enzymes* (2nd ed.), Boyer, P. D., Lardy, H., and Myrback, K. (eds.), Academic Press, Vol. 1:619)



- [] = upper limit of penicillin resistance in units per ml.
- () = molecules of penicillinase per cell.
- M = mutation.

*In *B. cereus* mutations have been observed which affect penicillin resistance level, rate of formation of penicillinase, and inducibility of enzyme synthesis by penicillin. These properties vary independently, indicating that each of them is under control of a mutationally different genetic element.

TABLE 11.3
Classes of Mutants at the *lac* Locus in *E. coli*

$i^+ z^+ y^+$	wild type; inducible formation of β -galactosidase and galactoside-permease
$i^- z^+ y^+$	constitutive mutant; β -gal. and gal.-permease always present
$i^+ z^+ y^-$	cryptic mutant; β -gal. inducible, permease never present
$i^- z^+ y^-$	cryptic-constitutive; β -gal. always present, permease never present
$i^+ z^- y^+$	β -gal. never present, permease inducible
$i^- z^- y^+$	β -gal. never present, permease always present
$i^+ z^- y^-$	neither β -gal. or permease is ever found
$i^- z^- y^-$	neither β -gal. or permease is ever found
$i^+ o^c z^+ y^+$	partially constitutive when o^c is in <i>cis</i> position with other genes
$i^+ o^o z^+ y^+$	neither β -gal. or permease is inducible (mimics deletion)

been described, which vary in their basal enzyme content and response to inducers; they are listed in Table 11.3. The letter *z* refers to the genetic capacity of the strain to make β -galactosidase, *y* refers to galactoside-permease, and *i* refers to inducibility. It is evident that here, as in the penicillinase system, the ability to make the enzyme is separable from its inducibility.

The genetic significance of so-called constitutive mutants can hardly be overstated, for they have provided the first direct evidence that the property of inducibility-repressibility is under genetic control. The model scheme outlined above included a parameter for gene control, in the R-moiety of the repressor, but did not specify how the gene might, in turn, be regulated. The fact that mutants arise that manufacture enzyme at a constant, inflexible rate shows that inducers and repressors are not essential elements of the enzyme-forming system, but rather that they are dispensable regulators. We may now formulate a new question: What is the nature of the constitutive mutation, and what light does it cast upon genetic mechanisms of regulation?

In tests both by recombination and by transduction, the *y*, *z*, and *i* factors have been found to be closely linked in the so-called "*lac*" region of the *E. coli* chromosome. In a study of interactions among these factors, crosses were made of various mutants and measurements of β -galactosidase were performed on the resulting zygotes. The results given in Figure 11.16, were obtained with zygotes from the cross:

$$Hfr z^+ i^+ Str-s T6-s \times F^- z^- i^- Str-r T6-r \quad (\text{cross 1})$$

When the *Hfr* chromosome, carrying $z^+ i^+$ enters the F^- cell which is $z^- i^-$, β -galactosidase formation begins at once, in the absence of added

inducer, and continues for some time, eventually stopping unless inducer is added. With the reciprocal cross (cross 2), however, no β -galactosidase whatsoever is formed in the absence of inducer. These results have been interpreted by Pardee, Jacob, and Monod, who did the experiments, to demonstrate that the i^- factor produces nothing which interferes with β -galactosidase formation, so that as soon as the z^+ factor enters, enzyme formation may begin. Since the z^+ and i^+ factors are closely linked, and virtually enter together, it must also be postulated that z^+ acts much faster than does i^+ . They propose that the i^+ factor produces a repressor of β -galactosidase, and that the reason why i^+ strains are inducible, is that inducer must be added to antagonize the preformed repressor. In the $z^+ i^-$ cells of cross 2, which are the F^- receptors, the cytoplasm is postulated to contain this repressor, and, consequently, the entrance of the $z^- i^-$ genes is of no avail. The results also show that i^+ is dominant to i^- .

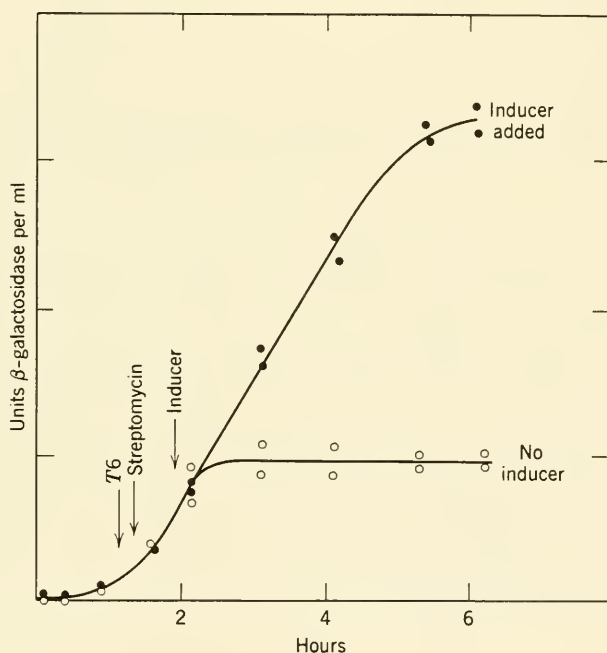


FIGURE 11.16. Formation of β -galactosidase in *E. coli* zygotes from the cross $Hfr z^+ i^+ \times F^- z^- i^-$. Enzyme formation begins as soon as the $z^+ i^+$ region enters the F^- cell, and persists for some time in the absence of inducer. Phage T6 and streptomycin are added to kill the *Hfr* cells (which are *T6s str-s*). In the reciprocal cross, no enzyme is formed in the absence of inducer (after Pardee, Jacob, and Monod, 1959, *J. Molec. Biology*, 1:165).

Support for this interpretation comes from further studies of cross 1, undertaken to elucidate the lag period before repressor formation begins. It was observed that blocking of protein synthesis by chloramphenicol or by 5-methyltryptophane did not change the rate of repressor formation, strongly indicating that the repressor is not a protein, or a product of protein synthesis in the zygote. It is suggested that the repressor may be a polynucleotide synthesized on the *Hfr* DNA after its entrance into the F^- cell.

An important property of the *i* gene product R_i is its capacity to determine the inducibility both of *y* and of *z*. As can be seen from Table 11.3, R_i is able to influence y^+ and z^+ independently, regardless of the state of the other factor. Furthermore, *y* and *z* need not be on the same chromosome as *i* for R_i to exert its effect. Crosses of the type: $Hfr\ z^+ i^- \times F^- z^- i^+$ give rise to zygotes which require inducer for the formation of the enzyme. Thus, the i^+ factor on one chromosome influences the action of z^+ on the other chromosome. This evidence does not clarify whether R_i acts at the chromosome level or in the cytoplasm (see p. 331).

Another class of mutants has been described which behave quite differently. They are the *o* mutants, which also map in the *lac* region. Mutants designated o^o appear to have neither β -galactosidase nor permease, nor repressor. They could be considered deletions for the entire region were it not for the fact that they are revertible to the wild-type state, in which all three activities reappear. Mutants designated o^c determine the constitutive synthesis of both enzymes, even in the presence of i^+ . However, the o^c element must be on the same chromosome as the factors which it influences. The simplest view of these findings is that repression and induction, under the influence of *o*, occur at the chromosome level, not at the site of enzyme formation. If this assumption is correct, the *o* gene exerts what might be called a position effect.

There are hints of similar phenomena in the literature of microbial genetics, e.g., at the *gal* locus in *E. coli* and in the histidine region in *Salmonella*, but none of them have yet been reported in detail. For a better insight into the possible significance and generality of these findings, it is necessary to consider these results in relation to other information about position effect.

POSITION EFFECT AND CONTROLLING ELEMENTS

We define position effect as the influence of one chromosomal region upon the expression of a neighboring region on the same chromosome. The first evidence of this totally unexpected phenomenon came from the

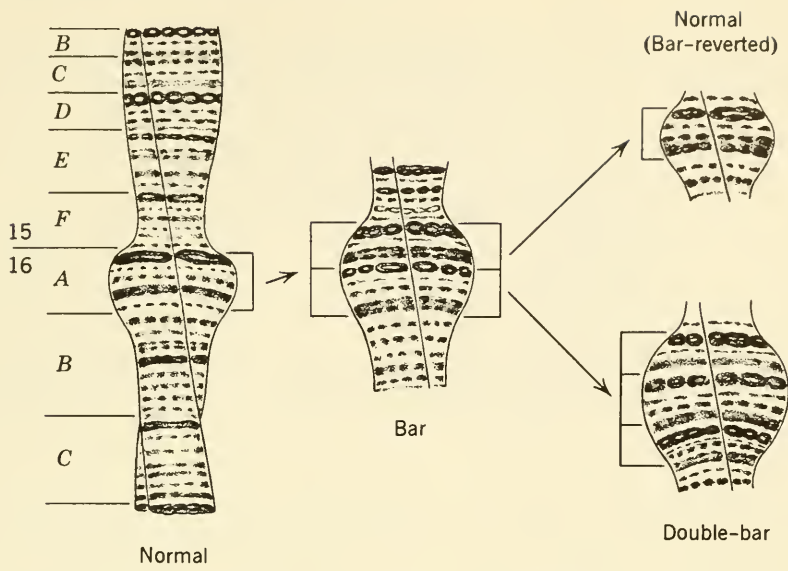


FIGURE 11.17. Comparison of chromosomal segments in Bar-eye and wild-type *Drosophila*. The segment 16A of the X chromosome consists of a characteristic banding pattern in the wild type, which is present twice in Bar-eye flies, and three times in double-Bar (from Bridges, 1936, *Science*, 83:210).

work of Sturtevant on Bar-eye, a dominant mutation on the X chromosome in *Drosophila*. Sturtevant showed that “mutations” from Bar-eye (*B*) to normal (*b*) and to an extreme form called double-Bar (*BB*) result from unequal crossing-over events. In a cytological study, Bridges found that the Bar phenotype results from repeats of the Bar region; it is present once in the normal, twice in Bar-eye, and three times in double-Bar as shown in Figure 11.17. Sturtevant compared the phenotypic effects of different numbers of repeats, (including another allele called *infra-Bar*), when located on the same and on homologous chromosomes. He counted the numbers of facets in the eye and found:

Genotype	Facets per Eye
$+/+$	780
$B/+$	358
B/B	69
$BB/+$	45
$B/infra-B$	73
$B\ infra-B/+$	50
$infra-B/infra-B$	293
$infra-B\ infra-B/+$	200

Clearly, when the Bar regions are together on one chromosome, there is a greater effect, i.e., a greater reduction in number of facets, than when each is on a separate chromosome. Sturtevant called this phenomenon position effect.

Until recently, the many examples of position effect could be grouped into two categories, stable or S type, and variegated or V type.

Study of the S-type effects led to the discovery of position pseudo-alleles, which have been discussed in Chapter 6. They reflect the large size of the functional gene, in contrast to the nucleotide dimensions of the units of mutation and recombination. Continuity of unaltered DNA along one chromosome appears to be necessary for the formation of a functional gene product. The *cis-trans* test of allelism demonstrates whether or not the mutated regions of two alleles are within the same functional region.

The V-type position effect was first found by Muller in 1927, in X-rayed *Drosophila*. When X-ray studies of *Drosophila* became popular in the 1930's, variegated position effects were widely observed and investigated by a number of workers. In general, this phenomenon involves chromosome rearrangement, with the insertion of the marker under observation into a new location, usually into a region considered to be heterochromatic (see p. 95). The change usually seen is the partial repression of the wild-type phenotype, as if something were interfering with the normal expression of the gene. The result is often a mottled appearance, most easily recognized in effects on pigmentation.

The example found by Muller was later shown to result from a translocation of the white locus (eye color). A remarkable feature of the phenomenon is that the heterochromatic region may exert a spreading effect along the chromosome, interfering with the expression of a number of linked loci, to extents which decrease with distance. The potency of the heterochromatin varies with its location, but the significance of this fact is not known.

One of the most spectacular examples of the spreading effect is shown in Figure 11.18, in which the region from band 3C₁ to 6A₂ of the X chromosome was inserted into a heterochromatic region of the fourth chromosome. Mottling was found for genes at both ends of the inserted piece, but not for the group which are in the center. Since the genes involved influence a wide variety of phenotypes, the response seems to be determined entirely by location.

That variegated position effects are reversible was first shown by Dubinin in Russia, and later by others. The method consisted essentially of selecting recombinants after crossing over, in which the translocated gene had been moved out of the heterochromatic regions.



FIGURE 11.18. Diagram of a *Drosophila* salivary chromosome showing the insertion of a segment of the X chromosome into the heterochromatic chromocenter region of the fourth chromosome (from Sutton, 1940, *Genetics*, 25:534).

When this occurred, the position effect was lost and the wild-type expression reappeared. Apparently then, the effect does not involve mutation as commonly understood, since it is fully reversible. A beautiful example of reversibility was provided by Catcheside in his analysis of a position effect at the *P* locus associated with a translocation in the plant *Oenothera*. By studying the movement of the *P* locus in and out of the translocation by crossing over, he correlated the instability of expression with the position of the locus.

Virtually no understanding of the V-type position effect is yet at hand, although many of the observations are almost 30 years old (which is very old in the field of genetics). The principal features of this phenomenon are: (1) the reversible repression of gene expression, and (2) the spreading effect.

About 10 years ago the first reports were published by McClintock of an intensive analysis of genic instability and the control of expression of one chromosomal element by another, in maize. Her studies have provided an opening wedge for a new attack upon the position effect phenomenon, and upon the more general problem which it reflects: genetic control devices operating at the chromosome level. In the brief account which follows, it will be impossible to do justice either to the analytical power of the experiments behind these results or, indeed, to the results themselves, which are complex, comprehensive, and diverse, and have not been published *in extenso*.

In the early stages of this investigation, McClintock discovered two chromosomal elements with extraordinary properties. One of them, called *Ds* (for dissociation) could induce chromosome breaks and mutations at any position in the chromosome where it was located, and could

be transposed from locus to locus, as judged by the change in the linkage relations of its effects. The other element was called *Ac* (for activator); its presence was required for expression of *Ds* activity, and the timing of *Ds*-induced events could be altered by the number of *Ac* elements present. *Ac* could also change its location and induce breaks and mutations and resembled *Ds* in these respects. They differed, however, in that *Ds* activity was strictly dependent on the presence of *Ac*, but *Ac* could act autonomously.

An astonishing diversity of phenomena appeared in this material which can be grouped in terms of (1) chromosome breakage, (2) transposition of controlling elements from one locus to another, (3) changes in gene expression, mimicking mutation, and (4) operational systems of controlling elements. The starting material included plants in which cycles of chromosome breakage had been occurring, and it is likely that this condition accounts for the origin of much of the variability. In later studies with this material, and in related investigations in other laboratories, it has become evident that chromosome breakage is not an essential feature of the basic phenomenon. In mutable systems not involving *Ds*, no breakage has been observed, and even with *Ds*, mutational and transpositional events occur in the absence of detectable breaks.

1. The location and the breakage effect of *Ds* were first recognized by the loss of dominant marker genes lying distal to *Ds* on the *Ds*-containing chromosomes, as shown in Figure 11.19. The timing of these breaks was controlled by the number of *Ac* alleles present. Increasing the *Ac* dosage resulted in later time of breakage, as shown by the decreased size of the colored sectors found. In the absence of *Ac*, no *Ds*-induced breaks occurred at all. In addition to showing dramatically the existence of some kind of metabolic control upon *Ds* exercised through *Ac*, this system was used to assay the *Ac* content of unknown material.

2. *Ds* was observed to undergo sudden shifts, called "changes of state," in which the *Ds*-induced events no longer involved breakage primarily, but more frequently (sometimes exclusively) changes in gene action, resembling mutations. Because of the high frequency of *Ds*-induced events, loci being influenced by *Ds* appeared mutable. Often the germinal mutations occurring under the influence of *Ds* were found subsequently to be stable, even in the presence of *Ac*. In such mutations, the *Ds* element was thought to have left the locus in question and, in numerous instances, the *Ds* activity reappeared at another site. McClintock has interpreted these findings in the most direct manner, as the physical transposition of the *Ds* element from one site to another.



FIGURE 11.19. Photographs of corn kernels demonstrating the effect of dosage of Ac upon the time of occurrence of *Ds*-induced chromosomal breaks.

(a) and (b). One Ac is present and the breaks at *Ds* occur early in development giving rise to large sectors.

(c). The pattern produced when two doses of Ac are present indicates the late time of occurrence of *Ds* breaks.

(d). When three doses of Ac are present, the *Ds*-induced breaks occur so late that only a few colored specks can be seen. In all plants used in these crosses, the genotype of the female parent was *C bz/C bz* and the male was *I Bz Ds* with *Ds* located between *Bz* and the centromere. *I* is a dominant inhibitor of pigmentation, allelic to *C* (from McClintock, 1951, *C. S. H. Symp. Quant. Biol.*, 16:13).

This view has been supported and confirmed in studies by Brink and colleagues, who reinvestigated the variegated pericarp system. R. A. Emerson, who initiated the study of mutable genes in maize, had investigated a P^{vc} (variegated pericarp) allele and concluded that the mutability was an intrinsic property of it. Brink *et al.* later showed that the mutable allele is a complex consisting of the top dominant allele P^{rr} and Mp . Mp is a controlling element very similar to Ac if not identical with it. The presence of Mp at the P locus suppresses the expression of P^{rr} , and mutation from variegated to stable red involves the transposition of Mp out of the P locus, frequently to a neighboring site, but occasionally to other chromosomes. The finding of just one Mp element in this material, before as well as after mutation and regardless of position, is strong evidence that Mp (and Ac as well) are physical entities which can move from site to site, rather than representing merely altered responses or activities.

The meaning of the transposition phenomenon is totally unclear at this time. It is not known whether mutability always involves a transposition, since there is no foolproof method for detecting transpositions in every system. However, this aspect of the findings does bear a formal resemblance to the following recent observations on episomes in the *E. coli* system.

As may be recalled from Chapter 5, in some strains of *E. coli*, the phage λ is present, in its prophage form, located close to the *gal* region. This region controls the formation of three enzymes involved in the metabolism of galactose, which are inducible and repressible. Whether λ is carried as prophage, multiplying with the chromosome, or whether it becomes vegetative, multiplying freely and lysing the cell, depends upon what has been called the repression of λ . The prophage state is considered a condition in which free phage multiplication is repressed. This repression can be lifted by UV-irradiation of the cells. In cells which are also repressed with respect to synthesis of the *gal* enzymes, UV-induction of λ also releases their repression, and there is a burst of enzyme formation in the cells before lysis. The specificity of the response is shown by the fact that induction of a different prophage, located further from the *gal* region, does not affect the *gal* enzymes.

Whether there are common features in mechanism which go beyond a formal similarity between the *E. coli* and maize systems in respect to release of repression by transposition is a problem for the future. There are other aspects of the maize system which may be of more direct relevance to the central theme of this chapter. These aspects concern the position effect itself, that is, the ability of controlling elements by their location to determine the expressibility of neighboring genes.

3. McClintock has described two kinds of controlling elements: those like *Ds* which influence only the locus at which they are inserted (they must be in the *cis* position), and require the presence of another controlling element elsewhere in the genome to activate them; and those like *Ac* which can act either on the spot, or at a distance, provided a suitably receptive site exists within the locus which is affected.

Both kinds of controlling elements can elicit a tremendous diversity of mutationlike responses. The reversibility of the phenotypes demonstrates that position effect is a more accurate description than mutation. Nonetheless, in the *Ds-Ac* system, if a locus is repressed by the presence of *Ds*, removal of *Ac* from the organism by outcrossing results in locking *Ds* into place, making its effect an irreversible one. Thus, mutations in the sense of changes in nucleic acids at the chemical level may be operationally indistinguishable from position effects.

McClintock's material was probably exceptional because of all the chromosome breakage involved in its origin. It must be borne in mind, however, that most of her observations have now been confirmed with other strains of maize, many of known pedigree. It is her view that the controlling elements which have gone out of kilter and, therefore, revealed themselves in her material, play a central role in normal metabolism and development. The recent findings of gene-controlled repression, in some cases apparently operating at the chromosome level (*o* genes and λ -*gal* induction in *E. coli*) support the view that position effects may be of great significance in wild-type systems. Presumably the position effects would not normally be exerted by transpositions or by phages, but rather by the action of cell metabolites upon susceptible sites which are permanently located within loci, and capable of exerting local position effect repression on neighboring gene sites.

4. Further insight into the remarkable properties of genes revealed in maize comes from the demonstration by McClintock that there are different systems of controlling elements, with particular alleles responding exclusively to one controlling element and not to others. As an example, let us consider the a_1 locus in chromosome 3, which influences pigment formation in seed and plant, and is epistatic to a number of other loci scattered through the genome.

Instability at the a_1 locus was first studied by M. M. Rhoades, who showed that it results from the presence of a gene called Dotted (*Dt*) in chromosome 9. When *Dt* is crossed into plants of the genotype $a_1 dt$, the a_1 locus loses its stability. A high frequency of somatic and germinal mutations occurs, giving rise to kernels with clones of pigmented cells (i.e., dots) on a colorless background (see Fig. 2.8), and to pigmented streaks on stems and leaves. The germinal mutations which

arise, to dominant A_1 , to intermediate phenotypes, and to a new a_1 , are subsequently stable in the presence of Dt . A dosage effect is seen, with additional Dt alleles increasing the number of mutations; but there is no effect on the time of mutation, comparable with the timing effect of Ac shown in Figure 11.19.

McClintock found that Dt did not substitute for either Ds or Ac in her material, but that other alleles at the a_1 locus, rather similar in phenotype to those described by Rhoades, responded to Ac but not to Dt . Subsequently, another group of alleles were found at the a_1 locus, which responded neither to Ac , Ds , or Dt , but to a different controlling element called Spm . Some features of the response were similar to what had been previously seen, but studies of Spm have also revealed a number of novel properties of which only one will be noted here. McClintock found that Spm could be present and active, or present and inactive. This important finding indicated for the first time in this material a lack of autonomy on the part of a controlling element, suggesting that its behavior is *determined*, not random. Further support for this view comes from a study in which an active Spm on one chromosome was combined by crossing with an inactive Spm located elsewhere in the other genome. In the resulting hybrids, both Spm elements were active, but when they were subsequently separated by outcrossing, in the next generation, the active and inactive elements again behaved as they had done initially. From this result one may suggest that the active Spm element conditions the cell in such a way that latent Spm elements are activated; presumably the activation is not purely at the chromosome level since the responding elements may be anywhere in the genome.

Further evidence on this point comes from the fact that the Spm and Ac systems each influence the behavior of many loci, although, for purposes of exposition, we have discussed only a_1 . When a number of different loci all responding to the same controlling element are combined in a plant, considerable uniformity is observed in the timing of mutational events. Presumably the controlling element determines the production of some substance which can activate the responding elements present at each of the mutating loci.

Another important aspect of the picture comes from studies of recombination within the a_1 region. It had previously been shown by Laughnan that intermediate alleles, for example, the pale allele a^p , could arise by some kind of recombinational event at meiosis. Subsequently, it was found possible to demonstrate recombination of the ability to respond to Dt . For instance, in the cross $A_1/a_1 \times a_1/a_1$, pale kernels recovered among the progeny resulting from recombination were found to differ in their response to Dt , some responding and others not.

This result indicates the segregation of a localized site determining receptivity to *Dt*. It seems likely that other localized sites exist in the a_1 region which determine response to other controlling elements like *Ac* and *Spm*, but there are no further data on this question as yet.

The prevalence and organized complexity of controlling element behavior in maize indicate strongly that these systems play a decisive role in normal, nonmutable material. Some hints about this role may come from comparisons with other systems. There are obvious parallels between the controlling elements of maize and those which regulate the rate of enzyme formation in *E. coli* and *Salmonella*. One similarity lies in the two-element control system, in which one element acts only in the *cis* position and is a part of the locus being affected, while the other element can be anywhere in the genome. The possibility that both an intranuclear signal and an "environmental" one, mediated in the cytoplasm, interact to determine the timing of activation of particular loci is consistent with observations made in both materials. However, the maize material has revealed a far greater complexity of intranuclear interaction than had been anticipated from any other studies, and underscores dramatically how much is still unknown about gene action.

Another phenomenon which shows evidence of genetic control of a repression mechanism concerns the formation of surface antigens in *Salmonella* and in *Paramecium*. These studies provide another approach to the analysis of genetic control devices, and are of particular importance because the antigens are proteins which can themselves be investigated.

In *Salmonella*, the genetic control of phase variation has been analyzed by Lederberg and Iino. There are two unlinked loci, H_1 and H_2 , which determine the specificity of the flagellar antigens. At any one time, only one of the two potential antigens is present, although the cell carries information for the synthesis of both. With a random frequency of 10^{-3} to 10^{-5} per cell division, a cell will switch from the antigen determined by one locus to that of the other. This phenomenon is phase variation. The question is: What determines which gene will be expressed and which repressed at any particular moment?

Employing transduction to study the effects of the phase of the donor and receptor cells upon the phase of the transduced progeny, a clear result was obtained. In transductions of the H_1 gene, only the phase of the receptor cell was relevant, and no influence of the donor H_1 gene upon its expression could be detected. The transduced H_2 gene, on the contrary, carried information dictating the phase of the transduced progeny as well as their antigenic specificity. When the expressed state of the donor H_2 was transduced, all progeny were expressed H_2 's

regardless of the phase of the receptors. When the unexpressed state of H_2 was transduced, the phase of the progeny was determined by the state of the receptor H_2 site.

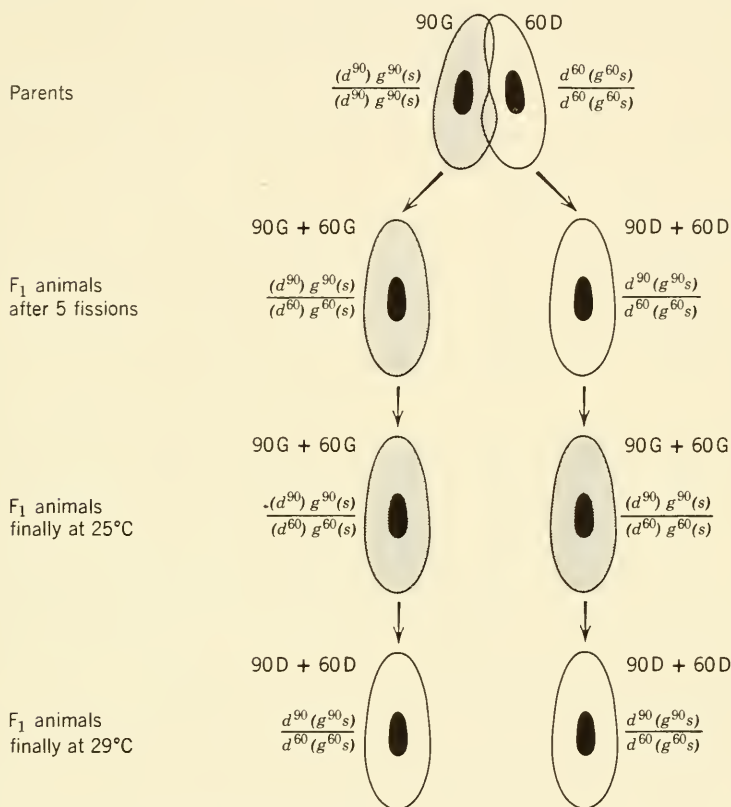
The inference seems well taken that the H_2 locus is able to repress the H_1 locus. One might say: when the H_2 locus turns itself on, it turns H_1 off. Thus, phase variation is an example of the controlling effect of one locus upon the expression of another. There are evident parallels between this system and some described by McClintock, but further data are required for a more detailed analysis.

In *Paramecium*, there are a wide diversity of antigenic types which have been investigated by Sonneborn and his students, and by Beale. *Paramecia* carry genetic information distributed at many different loci for the synthesis of a variety of surface antigens, only one of which is ever expressed at a time (except in heterozygotes to be discussed below). Presumably, a mechanism must exist not only for selection of the expressed antigen but also for repression of the formation of all the others. A great deal of discussion, speculation, and model building has been evoked by the well-demonstrated interplay of chromosomal and nonchromosomal influences in the determination of antigenic expression in *Paramecium*.

The genetic analysis developed by Beale of the antigenic changes induced by temperature provide an illustration of what is known at present about the genetic control of antigen formation. He found that the related stocks 60 and 90 behaved similarly in response to temperature changes. At temperatures of 18°C or lower, they developed S antigens. When transferred from low temperature to 25°C after a number of divisions, the animals changed their antigenic type from S to G and, similarly, at temperatures above 29°C, they switched to a different antigenic type noted as D. Although the antigens of the two stocks were very similar at each temperature, they could be distinguished by careful testing. A convenient feature of the system was the long delay (many divisions) after exposure to a particular temperature before the antigenic change was expressed. Thus it was possible to cross animals which had been grown at different temperatures.

It was established, first, that the antigens S, D, and G were each determined by a different unlinked gene, and that the small antigenic differences between stocks segregated as allelic differences. For example, in a cross of stocks 60 $\times$ 90, kept at 25°C throughout the experiment, the F_1 hybrids expressed both alleles by containing a mixture of the 60G and 90G antigens. At the next autogamy, the two alleles segregated, giving rise to pure 60G and pure 90G animals in a 1:1 ratio.

The relation between genetic determination of antigenicity and cytoplasmic determination of expression is shown graphically in Figure 11.20. Mating was carried out between animals of one stock grown at 25° C and therefore expressing their G antigen, and the other stock, grown at 29° C, expressing the D antigen. After conjugation, all F₁ animals whose cytoplasm came from a G parent, contained a mixture of 60G and 90G;



G & D refer to expressed antigens.

d, g, s refer to gene loci, with unexpressed genes in parentheses.

FIGURE 11.20. Inheritance of antigenicity in *Paramecium*. In a cross between animals of strain 90 grown at 25° C (and therefore expressing their g gene, 90G) and animals of strain 60 grown at 29° C (expressing their d gene, 60D) the F₁ progeny express antigens from both parents, and are therefore 60 + 90. Initially, the progeny inheriting cytoplasm from the G parent, are 60G + 90G while those from the D parent are 60D + 90D. Subsequently, all F₁ animals revert to the state determined by the temperature at which they are being kept (from Beale, 1954, *The Genetics of Paramecium Aurelia*, Cambridge, The University Press).

and all F_1 's whose cytoplasm came from the D parent, were a mixture of 60D and 90D. Thus, the new allele which entered at conjugation was expressed in a few fissions, but whether the *g* gene or the *d* gene was chosen depended on the prior state of the cytoplasm. After many divisions, those F_1 animals maintained at 25° C all developed the G antigens; and genetically identical animals kept at 29° C all developed D antigens. This beautiful experiment demonstrates dramatically the specific contributions of gene and cytoplasm in determination of antigenic type.

Now let us compare these results in a formal way (ignoring the many biological differences between the systems) with phase variation in *Salmonella*. In both systems, more antigenic potentialities exist in the organism than are expressed at any one time, with each potentiality determined by a different gene. Consequently, in both systems a mechanism is necessary for choosing among alternatives. The *Paramecium* analysis demonstrates that this choice operates *via* the cytoplasm, but reveals nothing about the origin of the cytoplasmic differences. The *Salmonella* analysis demonstrates the involvement of a chromosomal component in the choice between expression of the H_1 and H_2 genes, but presumably requires that the effect of this element at the H_2 locus be mediated *via* the cytoplasm for it to reach the H_1 locus. Conceivably, the two methods of analysis have uncovered two aspects of the same mechanism, there being both a genetic and an environmentally determined component involved in both systems. This suggestion is related to the speculations (p. 331) about the bipartite nature of repressors, one part being of direct genetic origin, the other part a metabolite. If the rate-limiting factor in antigenic change is the metabolite component, as may be likely in the temperature system in *Paramecium*, the existence of a genetic controlling element could not be detected.

Much of the discussion in the literature has centered on the possible nature of the cytoplasmic component elicited by the environment, which seems to have the dual role of inducing the formation of one protein and repressing formation of all the others. There has been no evidence whether the choice occurs at the chromosome level or at the level of protein formation, but the prevalent view has been the latter. On the controlling-element hypothesis, these are not mutually exclusive alternatives. A controlling element might activate one locus and inactivate another with respect to production of a gene product (e.g., part of a repressor) which itself acts at the level of protein synthesis. In an "ordered" system like antigen determination in *Paramecium*, (in contrast to the "disordered" systems of maize and *Salmonella* involving high spontaneous mutability), an additional concept is required, namely, a feedback from the cytoplasm to activate the controlling element.

It must be evident to the reader how remote many of the biological

systems under investigation are from the operating molecules in whose name the interpretations are drawn. It should also be evident, however, that the direction of modern biology is towards systems which will give answers to biological questions, and yet be interpretable at the molecular level. This goal is not often reached, and in some lines of research hardly attempted. Of course, a hazard of the analytical approach is that by focusing upon elements of a system, one may altogether miss the over-all view. An example of a line of research which appears to be very promising in terms of over-all view rather than analytical detail is that of nuclear transfer.

NUCLEAR TRANSFER

Within recent years, the technique has been developed of transplanting intact nuclei from one cell to another in amphibian oöcytes and early embryos. Owing primarily to the pioneering efforts of Briggs and King, it has become possible to explore the changes, if any, occurring in nuclei during embryonic development.

The method consists essentially of enucleating an oöcyte (they used the frog *Rana pipiens*) and transferring into it a nucleus from some particular cell of a developing embryo. In the initial experiments, the transferred nucleus was derived from an early blastula stage only a few divisions removed from the original diploid nucleus of the fertilized egg. Techniques were developed to such a remarkable state that 40–80 per cent of the resulting oöcytes underwent normal development, giving rise finally to tadpoles.

Using these results as a control, Briggs and King compared the behavior of these nuclei from early blastulae with nuclei obtained from a number of specified regions of embryonic development during succeeding stages. In brief, they found that nuclei from increasingly differentiated cells lost their capacity to determine normal development after transfer. The problem then arose how to interpret these results. Are the nuclei becoming altered *in situ* before transfer, or are they merely more susceptible to injury?

The experimental approach to this question was derived from microbiological methodology: to determine whether or not the alterations observed were stable upon subculture. With microorganisms one follows the stability of a phenotype by observing the descendants of a particular cell, i.e., a clone, through many generations. A kind of cloning was developed with nuclear transfer experiments. First, nuclei from some chosen stage of development were transferred to oöcytes, which were allowed to develop to early blastula, that is, for some 12–14 divisions.

Then one or several of these blastulae were chosen, and groups of nuclei from each were transferred to oöcytes, and the process was repeated. At each "subculture," most of the material was allowed to develop as far as it would go in order to score the phenotypes, and only a few blastulae were sacrificed for further cloning. Similar studies have been carried out with the related genus, *Xenopus*, with similar findings.

There is much greater homogeneity of developmental stage reached in the subclones than in the original set of nuclei taken from the late stages of development. For example, when nuclei are taken from various regions of late gastrulae of *Rana pipiens* (such as chorda mesoderm, presumptive medullary plate, or endoderm), most of them give rise to embryos which are blocked at early stages even before gastrulation; but quite a range of response levels have been observed. The group of embryos which develop by clonal subculture from one of these nuclei, on the contrary, are fairly uniform in distribution of stages to which they develop. Similarly, the group of embryos developing from a different one of these original transferred nuclei is quite uniform, but there may be drastic differences in level of development between the two sets (Figure 11.21).

These results demonstrate the existence of great heritable stability on the part of the alterations experienced by the original nuclei taken from late embryonic stages. These alterations, which differ from nucleus to nucleus, are transmitted regularly in subsequent nuclear divisions.

It is evident that little information is as yet available on the origin of the initial alterations in nuclei from late embryonic stages. Nonetheless, the stability of these alterations under subsequent cloning conditions is a very important finding in its own right.

From the analytical viewpoint, this kind of experimentation has serious limitations, involving as it does a highly complex set of interacting variables. The transferred nucleus brings with it not only the chromosome complement but also the nucleolus, nuclear membrane, and attached bits of cytoplasm. Although the bulk of cytoplasm transferred is small, the possibility exists that it does not represent a random sample, but rather a region of specialized function attached to or adjacent to the nucleus. The results cannot be fully accepted as evidence of chromosomal effects, nor even of nuclear effects, without correlated evidence from an independent line of experimentation.

CONCLUDING REMARKS

In this chapter we have examined a few of the current lines of research which have been particularly fruitful in providing new information, new

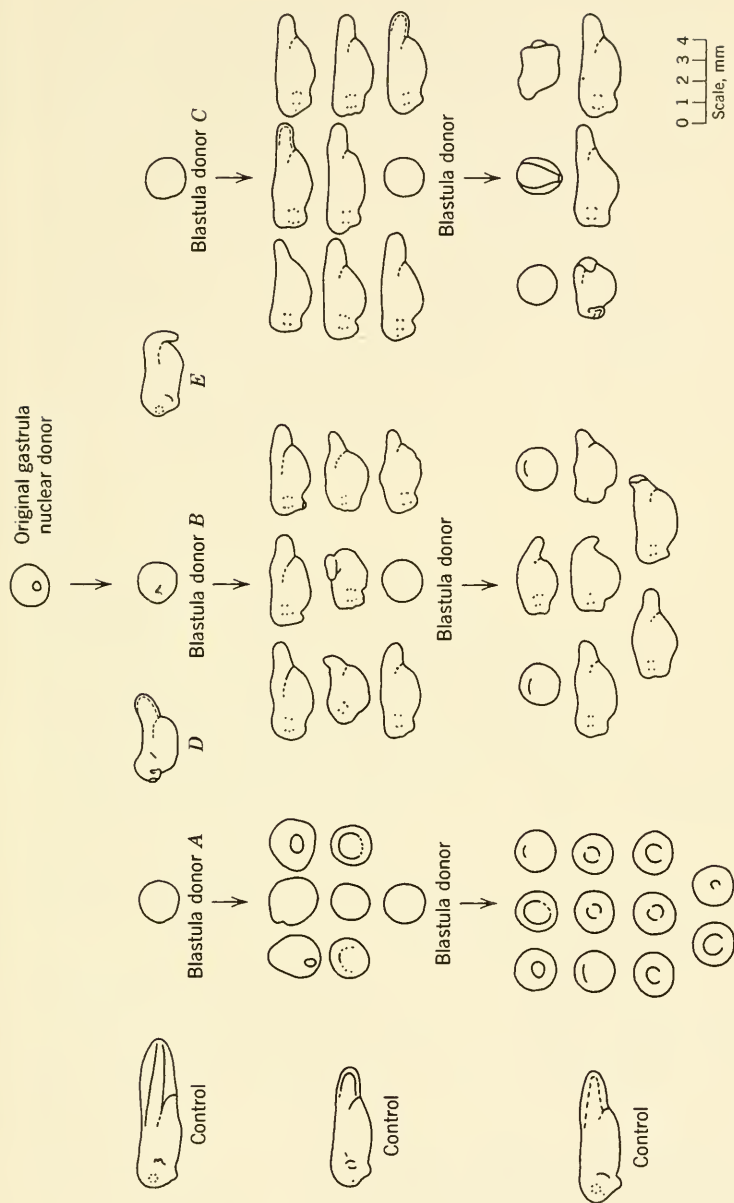


FIGURE 11.21. Serial transplantation of endoderm nuclei from late gastrulae of *Rana pipiens*. All three nuclear clones were derived from a single donor. Descendants of nucleus A developed only to gastrula stages, in both first and second subculture generations. Descendants of nuclei B and C developed to abnormal postneurula stages. No evidence was found of recovery towards more normal development following subculture. D and E are examples of the potentialities of similar blastulae which received nuclei from the original gastrula, and were allowed to complete their development (from King and Briggs, 1956, *C. S. H. Symp. Biol.*, 21:271).

concepts, and new experimental approaches to the understanding of genetic control mechanisms in cell integration. We have seen that these investigations range from the analysis of the replication of nucleic acids and the synthesis of proteins in defined *in vitro* systems to the transfer of whole nuclei from cell to cell. All the combined knowledge of biological and physical sciences is being drawn upon to devise experiments which will reveal the dynamic interactions among cell parts.

In this effort, it is now beginning to appear that the regulatory role of genetic material is of central importance. The ability to study the genetic control of repression and regulation of enzyme formation in wild-type cells as well as in mutants has provided both experimental approaches and concepts which will undoubtedly be of wide applicability in all organisms. The concept of rate-controlling genes is a very old one in genetics, first studied and discussed extensively by Goldschmidt in the 1920's. It is much more available to analysis in its present form, in which it can be pursued at the specific level of enzyme synthesis.

BIBLIOGRAPHY

- Allfrey, V. G., J. W. Hopkins, J. H. Frenster, and A. E. Mirsky, 1960. Reactions governing incorporation of amino acids into the proteins of the isolated cell nucleus. *Ann. N. Y. Acad. Sci.*, **88**:722-740.
- Beermann, W., 1956. Nuclear differentiation and functional morphology of chromosomes. *C. S. H. Symp. Quant. Biol.*, **21**:217-232.
- Brink, R. A., 1958. Mutable loci and development of the organism. *J. Cell. Comp. Physiol.*, **52**:169-195.
- Cohen, G. N., and J. Monod, 1957. Bacterial permeases. *Bact. Rev.*, **21**:169-194.
- Cohn, M., and J. Monod, 1953. Specific inhibition and induction of enzyme biosynthesis. In: Davies, R. and E. F. Gale (eds.), *Adaptation in Micro-Organisms*. Cambridge, the University Press.
- Gorini, L., and W. K. Maas, 1958. Feedback control of the formation of biosynthetic enzymes. In: McElroy, W. D. and B. Glass (eds.), *The Chemical Basis of Development*. Baltimore, The Johns Hopkins Press, pp. 469-478.
- Gorini, L., 1960. Antagonism between substrate and repressor in controlling the formation of a biosynthetic enzyme. *Proc. Natl. Acad. Sci. Wash.*, **46**: 682-690.
- Hämmerling, J., 1953. Nucleo-cytoplasmic relationships in the development of *Acetabularia*. *Intl. Rev. Cytol.*, **2**:475-498.
- Hogness, D. S., 1959. Induced enzyme synthesis. In: Oncley, J. L. (ed.), *Biophysical Science*. New York, Wiley, pp. 256-268.
- King, T. J., and R. Briggs, 1956. Serial transplantation of embryonic nuclei. *C. S. H. Symp. Quant. Biol.*, **27**:271-290.

- Lederberg, J., and T. Iino, 1956. Phase variation in *Salmonella*. *Genetics*, **41**:743-757.
- Lewis, E. B., 1949. The phenomenon of position effect. *Adv. Genet.*, **3**:73-115.
- McClintock, B., 1956. Intranuclear systems controlling gene action and mutation. *Brookhaven Symp. Biology*, **8**:58-74.
- Monod, J., and M. Cohn, 1952. La Biosynthèse induite des enzymes. *Adv. Enzymol.*, **13**:67-119.
- Pavan, C., 1959. Morphological and physiological aspects of chromosomal activities. *X Intl. Congr. Genet.*, **1**:321-336.
- Pardee, A. B., 1959. The control of enzyme activity. In: Boyer, P. D., H. Lardy, and K. Myrbäck (eds.), *The Enzymes* (2nd ed.), Vol. 1. New York, Academic Press, pp. 681-716.
- Pollock, M. R., 1959. Induced formation of enzymes. In: Boyer, P. D., H. Lardy, and K. Myrbäck (eds.), *The Enzymes* (2nd ed.), Vol. 1. New York, Academic Press, pp. 619-680.
- Rhoades, M. M., 1941. The genetic control of mutability in maize. *C. S. H. Symp. Quant. Biol.*, **9**:138-144.
- Sonneborn, T. M., 1960. The gene and cell differentiation. *Proc. Natl. Acad. Sci. Wash.*, **46**:149-165.
- Szilard, L., 1960. Control of the formation of specific proteins in bacteria and in animal cells. *Proc. Natl. Acad. Sci. Wash.*, **46**:277.
- Vogel, H. J., 1960. In: Bonner, D. M. (ed.), *Control Mechanisms in Cellular Processes*.
- Waddington, C. H. (ed.), 1959. *Biological Organization, Cellular and Sub-Cellular*. London, Pergamon Press.
- Zalokar, M., 1960. Sites of protein and ribonucleic acid synthesis in the cell. *Expt. Cell Res.*, **19**:559-576.
- Zamecnik, P. C., 1960. Historical and current aspects of the problem of protein synthesis. *Harvey Lect.*, **54**:256-281.

12

Heredity in Somatic Cells

Somatic cells are those that make up the body or the soma of multicellular organisms. They are distinct from the gametes and their immediate progenitors, which comprise the germ cells. The somatic cells contained in the various tissues of the body are found to be structurally, metabolically, and chemically different. Yet they all arise from the single fertilized zygote which contains all of the genetic information used for the differentiation that produces, during development, the distinguishable cell types with such a harmony in time and space. Each type is usually represented by considerable numbers of cells which can produce only their own kind or, when differentiation is incomplete, a restricted number of kinds. Each differentiated somatic cell thus comes to have a stable heredity characteristic of its type. This stability is reflected even when somatic cells are removed from the body and cultured. Although the environment in which it is found determines within limits its condition at the moment, a somatic cell rarely escapes its hereditary destiny and progresses in a different direction. But clearly it is these rare escapes—these aberrant behaviors—which may provide a key to the processes of hereditary variation in the somatic cell. Both normal and abnormal development have their particular embryological and pathological aspects, at the same time remaining basically problems of cell heredity.

NUCLEAR DIFFERENTIATION

Do the nuclei change in the course of differentiation in accord with the variety of cell types found? Clearly nuclei are critical for develop-

ment because cells from which they have been removed cannot multiply and are at best moribund. But is the nucleus the primary site of the changes that transform the egg into the multifarious cells that make up the soma? It was proposed by the great German biologist, Weissmann, that mitoses gave rise to qualitatively different nuclei; he supposed that this segregation of nuclear elements gave rise to the differences we see among somatic cells. On what grounds was this provocative hypothesis discarded? In many organisms, especially animals, the germ cells can be distinguished early from the somatic cells. In the extreme and classical example of the roundworm, *Ascaris*, the nuclei of the germ line receive replications of all chromosome material of the fertilized egg, whereas the somatic nuclei lack the ends of the original chromosomes eliminated during cleavage (Figure 12.1). Apparently the germ line chromosomes are aggregates, and many of the component chromosomes are unnecessary for the development of the soma. Such *supernumeraries* are frequently found.

Nonetheless, no similar differentiation of chromosomes has been found to distinguish the various types of somatic cells from one another. In *Ascaris* the aggregate of supernumeraries enables the identification of the germ line, but even in the soma, once the chromosome complement is established, it remains constant. In other organisms where elimination of chromosomes does not occur, the first, and each succeeding, mitosis in the fertilized egg results in the production of what seem to be identical sets of chromosomes. But, although they appear the same, it does not

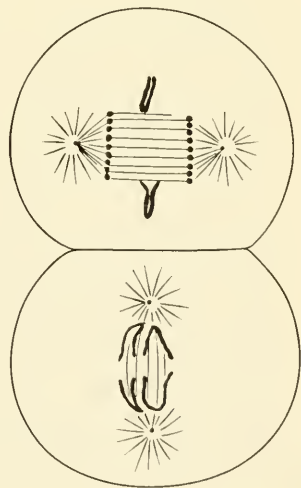


FIGURE 12.1. Chromosome elimination at the second cleavage of the egg of *Ascaris* (after Boveri, in: Ries and Gersch, 1953, *Biologie der Zelle*, Leipzig, B. G. Taubner Verlagsgesellschaft).

necessarily follow that they possess identical genotypes. This possibility cannot be examined by breeding tests because the cells in question are irreversibly determined as somatic cells. Such tests could be made with organisms whose germ and soma are not irreversibly differentiated. It is difficult to be sure that an organism meets this criterion because there is always the possibility that the sexual cells actually arise from progenitors that have remained embryonic although they are surrounded by differentiated neighbors. The same reservation must be held about studies on the regeneration of differentiated parts of plants and animals. In unicellular forms, such as the ciliate protozoa and certain algae like *Acetabularia*, differentiation is reversible with no irreversible genetic change as a consequence. In some animals it has been shown that different regions of the cytoplasm of the egg are irreversibly determined as to their subsequent fate *before* the nucleus divides. Thus something other than nuclear differentiation must be responsible. In the classical experiments that overthrew the theory of Weissmann, another German, Spemann, showed that the nuclei of the developing egg of the salamander, at least until the 16-cell stage, retain their ability to direct the formation of a perfect embryo.

The gonads of animals, themselves highly differentiated, provide another test. By the application of hormones, sex reversal can be achieved in, for example, the medaka fish. Genetically female cells thus form sperm, and vice versa. But breeding tests show that such sex-reversed sperm have a female genotype and the reversed eggs, a male genotype. Despite the externally directed change in the course of differentiation, the genetic constitution, insofar as it was testable, was unchanged. Thus, the answer to our question about nuclear differentiation seemed an emphatic "no," and we were confronted with a paradox: heritable changes occur during development in cells whose nuclei are the same.

We now know that this is not always true and also that the paradox is anyhow resolvable. Transplantation of nuclei in the frog's egg shows that irreversible nuclear changes do occur, not only during normal development but also after residence in foreign cytoplasm. This suggests that extranuclear factors may control somatic cell heredity through their influence on nuclei. No chromosomal changes are apparent in these cases, but in the dipteran insects, in certain tissues at certain times during development, specific crossbands of the polytene chromosomes enlarge into puffs (see Figure 11.7). In other types of cells at the same time, different regions of the chromosome are puffed; in the same type of cells at different times, different regions puff. These spatially differentiated sequellae could be the morphological expression of the pattern of gene states in time and space that brings about cell differentiation.

Nothing similar has been observed in other chromosomes, perhaps for the reason that they are not as easy to observe.

If these phenomena are correctly interpreted and of general occurrence, there is still the question of how they are controlled. Whether some extrachromosomal condition brings about this chromosomal behavior, or whether it is achieved by the action of chromosomal controlling agents, we cannot say. But we do know, as should be evident from the contents of the earlier chapters in this book, that there are a number of other ways in which somatic variation may be achieved. Mutation of chromosomal genes and selection, mutation and segregation of nonchromosomal factors with autonomous action, mechanisms of infectious heredity including viruses, and the formation of phenocopies, all provide food for thought and experimentation. Perhaps it will be shown that some of these processes do not have the orderliness necessary for organized development; they are still of interest as possible explanations of abnormal growth and the degenerative changes accompanying senescence. But even more important, their study will reveal the hereditary mechanisms which operate in somatic cells, in terms of which problems of normal and abnormal development must find their solution. Each of these mechanisms will be taken up in turn; first we will deal with a phenomenon of genetic exchange in somatic cells, alternatively called *somatic* or *mitotic crossing over*.

RECOMBINATION IN SOMATIC CELLS

It was long ago shown that genetic exchange can occur during mitosis by the process of crossing over. In making his beautiful demonstration of this fact, Curt Stern used *Drosophila*, heterozygous for the recessive genes singed (*sn*), which curls the bristles, and yellow (*y*), which colors the cutaneous tissue yellow instead of grey. These genes are both located on the X chromosome, as shown in Figure 12.2. The heterozygous flies showed mosaic patches of different sorts. The most frequent was a twin spot, in which a small patch of yellow tissue bordered on a patch with singed bristles. The next most frequent mosaic showed only a small patch of yellow tissue in the midst of a background of wild-type cells. Finally, in low frequency, mosaics with single singed-bristle spots were found. These results are explained in terms of an exchange between the homologous chromosomes, which can in fact be observed to be paired during the prophase of mitosis in the somatic cells of *Drosophila*. The centromeres of the four chromatids involved in the exchange later separate in the usual mitotic fashion, *a* and *b*, like *c* and *d*, moving

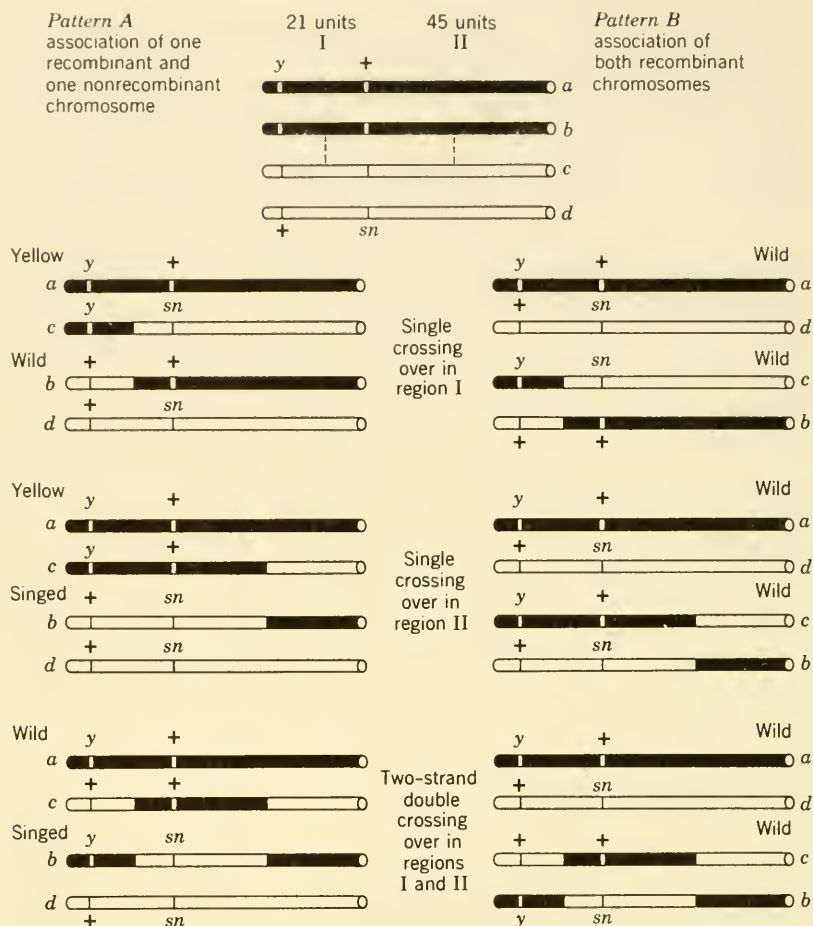


FIGURE 12.2. Somatic crossing over in the X chromosome of *Drosophila* (after Stern, 1936, *Genetics*, 21:625).

to opposite poles. Unlike meiosis, this process leads to no reduction in number.

The possibilities for chromosome separation are shown in Figure 12.2; only one arrangement of four will give rise to homozygosity of a given recessive gene after each kind of crossing over. If the chromosomes separate in pattern A after crossing over in region II, daughter nuclei result, each one homozygous for a different locus, and twin spots are a consequence. The size of these spots, which is not random, is a function of the time in development when somatic crossing over occurred and of the

rates of growth of the daughter cells. If crossing over occurs in region I, a single yellow spot would be expected. Since the genetic distance between *y* and *sn* is smaller than that between *sn* and the centromere, homozygosity for *y* alone would be rarer than homozygosity for both genes. Homozygosity for *sn* would be achieved by double crossing over in regions I and II, and the extreme rarity of single singed spots is in accord with expectation. Somatic crossing over is another instance where the reciprocal products of one exchange can be recovered, allowing one more demonstration that crossing over occurs in the four-strand stage.

The existence of twin spots shows that the characters *y* and *sn* are autonomous in development; neither type of cell prevents the characteristic expression of the other, despite their adjacent location in small patches. This is frequently but not always the case. In *Drosophila* some individuals are gynandromorphs, female on one side of the body and male on the other. This is due to the loss of an X chromosome from one of the two cells produced by the first division of an XX zygote. The cell derivative that is XX in genotype forms female tissue; that which is X forms male (Figure 12.3). Apparently in *Drosophila* intracellular factors determine sex and the eosin eye-color character as well. But certain eye pigments are under hormonal control. A gynandromorph in which the female tissue is heterozygous for vermilion eyes, *+/v*, and where the male tissue has only the *v* gene, does not show a vermilion eye on the male side. This situation was brilliantly explored by Beadle and Ephrussi, who began their studies of this problem in Paris. They implanted the embryonic eye buds from vermilion larvae into the body cavities of wild-type larval hosts where the implants received a substance that caused the eyes to develop normal pigmentation. It was eventually shown that the diffusible hormone was kynurenine, and further work has



FIGURE 12.3. A gynandromorph of *Drosophila melanogaster*. The left side of the head and thorax is wild type and female; the right side is eosin, forked and male (except for a patch of wild type tissue in the right eye) (from Sturtevant and Beadle, 1939, *An Introduction to Genetics*, Philadelphia, Saunders).

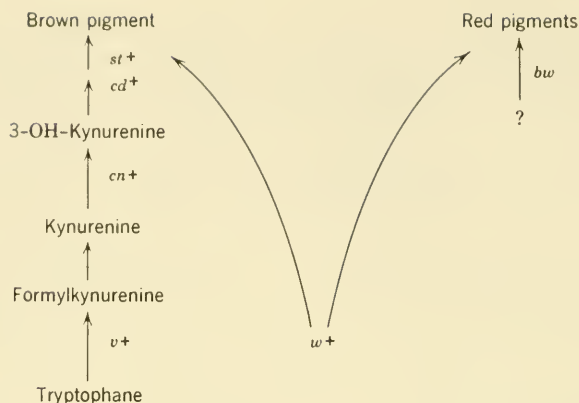


FIGURE 12.4. The biochemical basis of the genetic control of eye color in *Drosophila* (from Wagner and Mitchell, 1955, *Genetics and Metabolism*, New York, Wiley; after Beadle, 1945, *Chem. Rev.*, **37**:15).

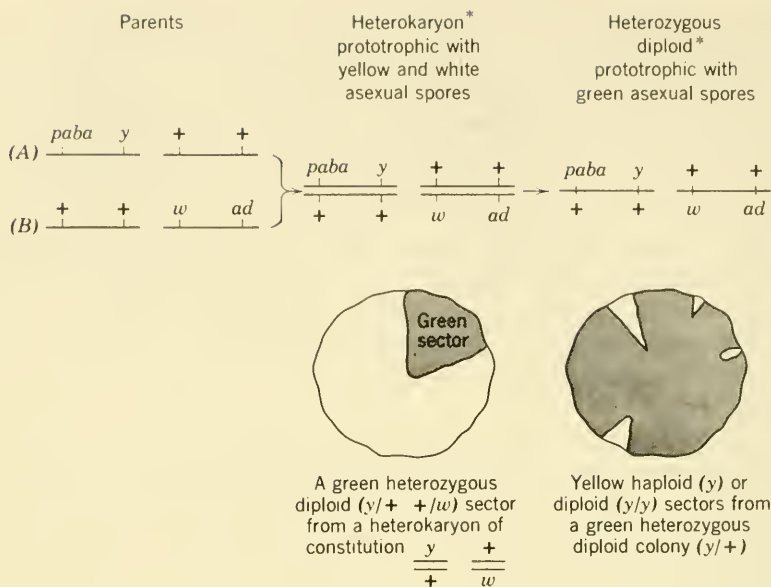
established this and other substances in sequence as precursors of the brown pigment in the insect eye (Figure 12.4).

The reparable versus irreparable nature of lethals has also been investigated in *Drosophila* with the use of somatic crossing over with the same *y* and *sn* genes shown in Figure 12.2. If, for example, there were a lethal gene adjacent to the yellow marker on the upper chromosome, and if twin spots regularly occurred, it would be concluded that the lethal does not prevent the survival of a patch of cells homozygous for it as long as the rest of the animal remains heterozygous. On the other hand, if single singed spots were of regular occurrence, we would know they were the survivors of segregations that would have led to twin spots except that the yellow-lethal homozygote did not survive. Demerec found with the use of this method that there are both kinds of sex-linked lethals. Most lethals that are demonstrated to be deficiencies are also autonomous and are cell lethals, but those that survive when present on the single X of the male make for viable cells. Some deficiencies are so restricted in scope as to not include an irreparable functional unit that even haploid "organisms" such as phages survive their presence.

Somatic crossing over is not restricted to the X chromosome of *Drosophila*. It occurs between other chromosomes and in other organisms, such as yeast and fungi. An extensive analysis of the process has been carried out by Pontecorvo and his associates in Glasgow with the fungus, *Aspergillus nidulans*, where the products of somatic crossing over can be recovered and analyzed. This organism contains haploid nuclei and pro-

duces uninucleate, asexual pores. When these germinate, hyphae and eventually a mycelial mat are formed, as in *Neurospora*. The hyphae of two genetically different strains may fuse together and contribute their nuclei to a common mycelium. Since the cells making up this mycelium have many nuclei of both genotypes in a common cytoplasm, the new strain is a heterokaryon. A heterokaryon of *Aspergillus nidulans* forms asexual spores which are not heterokaryotic. Each contains a single nucleus of one or the other type, with the result that strains resembling the parents can be re-established. A stable heterokaryon can be formed by mixing two strains with alternate nutritional requirements. In this event, the two types of nuclei in a common cytoplasm complement one another, allow the synthesis of the required substances, and enable growth on minimal medium.

A heterokaryon between, say, a yellow para-amino benzoic acidless (*y paba*) and a white adenineless (*w ad*) strain possesses nuclei differing both in the chemical site of pigment production and the growth factor required. It will form a colony on minimal medium but, when spores develop on the colony surface, they will not contain the wild-type pigment because they are uninucleate, and spore color is an autonomous character. But once in a while the colony will show a sector with green wild-type spore pigmentation (Figure 12.5). Individual uninucleate spores isolated from such a sector will grow on minimal medium and develop into uniformly green colonies. These spores turn out to be diploid and heterozygous for the parental chromosomes. Complementation took place between the w^+ and y^+ genes for pigment production and allowed identification and selection of the diploid tissue. Alternatively, diploids may be selected by plating the asexual spores from such a heterokaryon onto minimal medium where only those with diploid nuclei will form colonies with, of course, green pigmentation. Such selective procedures allow the frequency of diploids of spontaneous origin to be estimated at 10^{-6} ; when a heterokaryon is exposed to ultraviolet light, this frequency may be, for unknown reasons, raised to 10^{-2} . Although chromosome counts are difficult in *Aspergillus*, the diploid nature of these spores may be ascertained in a number of ways: their volume and DNA content are twice those of haploid spores, and the parental genes can be recovered after meiosis. *Aspergillus*, unlike *Neurospora*, does not have two mating types but any strain can form fertile fruiting bodies by itself. Within these structures any of the nuclei present may fuse to form a zygote nucleus which promptly undergoes meiosis. In the fruiting body of the diploid strain when two nuclei fuse, a tetraploid zygote is formed; this, at meiosis, gives rise to some haploid progeny, among which combinations of the parental genes are represented.

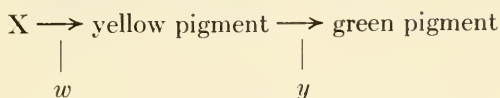


* When the two chromosomes $\frac{paba \quad y}{+ \quad +}$ and $\frac{+ \quad +}{+ \quad +}$ are in different nuclei in a heterokaryon, the genotype is written $\frac{paba \quad y}{+ \quad +}$; when the two chromosomes are in the same diploid nucleus, the genotype is written $\frac{paba \quad y}{+ \quad +}$

FIGURE 12.5. Sected colonies of *Aspergillus*.

The presence of both parental chromosome sets in the diploid nucleus can also be demonstrated by somatic recombination and a process of chromosome reduction which cause heterozygous diploid colonies to show sectors for color. For example, heterozygotes from a heterokaryon between yellow (y) and white (w) auxotrophs of this composition, $\frac{paba \quad y \quad + \quad +}{+ \quad + \quad w \quad ad}$, form yellow and white sectors that are no longer heterozygous for color markers and sometimes not for auxotrophic markers either (Figure 12.5). About 15 per cent of these sectors give rise to haploid conidia. When white haploid patches are selected, half are of genotype $paba \quad y \quad w \quad ad$ and half are $+ \quad + \quad w \quad ad$, showing an independent behavior of the two chromosomes in the reduction process. When yellow haploid patches are selected, they are all $paba \quad y \quad + \quad +$. The alternate

type, *paba y w ad*, does not fall in this class; it is white rather than yellow because the white gene is epistatic to yellow:



The other 85 per cent of the sectors form diploid conidia which are homozygous for either *w* or *y*. The white diploid sectors are either Ad^+ or ad ; the latter are doubly homozygous, $\frac{w ad}{w ad}$, and the former, upon analysis of sexual spores from fruiting bodies, turn out to be recombinants of composition $\frac{w +}{w ad}$. The yellow diploid patches are either $\frac{+ y}{paba y}$ or $\frac{paba y}{paba y}$.

These homozygous conditions and the recombinations between the genes can be accounted for by somatic crossing over. Not only can the reciprocal recombination products be found in sectors corresponding to the twin spots of *Drosophila*, but both reciprocal products of exchange have been recovered from one nucleus. The experiment went like this. A heterozygote, which was heteroallelic at the ad_8 locus, was prepared and selection of adenine-independent asexual spores was made on minimal medium. Only two of the four possible products of an exchange between the two mutated sites at the ad_8 locus would be expected to be adenine-independent and to grow on the selective medium (Figure 12.6). By selecting haploid segregants from these adenine-independent strains, it could be discovered which contained the two crossover strands, *b* and *c*. This was possible because the ad_{16} allele allows growth on minimal medium at about half of the wild-type rate, while the allele containing the combination, $ad_{16} ad_8$, allows no growth at all. Thus, the possession of strands *c* and *d* could be distinguished. The presence in a haploid of strand *b* resulted in yellow color. The correctness of the identification of strand *c* was checked by crossing the haploid thought to contain it with wild type. Recombination during meiosis separated the allele, $ad_{16} ad_8$, into its components, $ad_{16}+$ and $+ad_8$. There can, therefore, be no doubt that somatic crossing over yields reciprocal products, which arise by exchange within a cistron.

Somatic crossing over can be used to map genes in cells that are diploid and not undergoing sexual reproduction. The principle is based on the fact that homozygosis can be achieved only if an exchange occurs between a locus and a centromere. Therefore, among homozygotes for a distal marker there should be some for markers between it and the

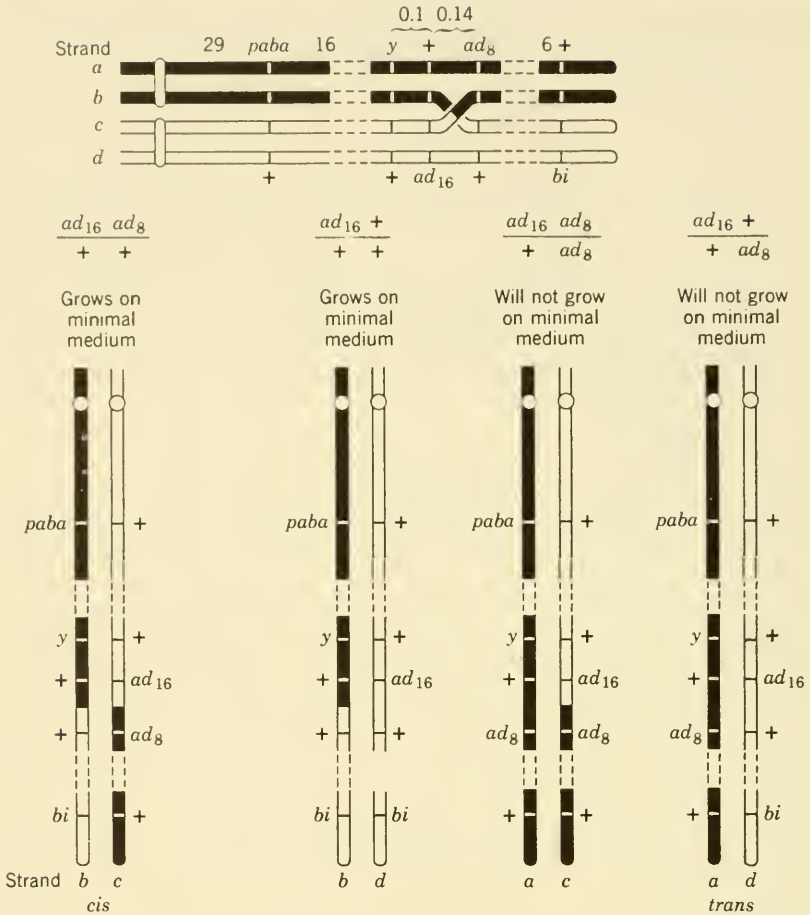


FIGURE 12.6. Recovery of reciprocal recombination products from mitotic crossing over in *Aspergillus*. The minimal medium in these experiments lacks adenine but contains biotin (adapted from Roper and Pritchard, 1955, *Nature*, 175:639).

centromere in inverse proportion to the proximity of the intermediate marker to that reference point. For example, if the order of genes is: centromere, *pro*, *paba*, *y*, there should be (barring double exchanges) no segregants homozygous for *pro* that are not at the same time homozygous for *paba*. Conversely, among the *y/y* homozygotes there should be more *paba/paba* than *pro/pro*. If *y* were proximal to the other two genes, then all *y/y* homozygotes should also be homozygous for *pro* and *paba*. When markers linked with the one used for selection are recessive and in

cis arrangement with it, the point of the exchange can be immediately deduced from the phenotype. For instance, if segregants homozygous for *y* are selected from a stock with the genotype $\frac{+ + +}{pro\ paba\ y}$

crossovers between *paba* and *y* will be only yellow in phenotype, crossovers between *pro* and *paba* will be yellow and *paba*-less, while crossovers between *pro* and the centromere will be yellow, *paba*-less, and prolineless. If the proximal markers are in *trans* arrangement with the selected marker, the position of the exchange must be ascertained by further analysis. It should be evident that the farther the selected marker is from the centromere, the more useful it will be for mapping the arm of the chromosome that contains it.

Table 12.1 shows a sample of data collected for mapping chromosome I. The centromere can be located by noting that point of change in the direction with which homozygosis for one marker is always associated with homozygosis for a series of others. In the example, this is between *an* and *pro*. The distances on the map in Table 12.1 are expressed as fractions of the total amount of exchange in each arm, which is set at 100. This is done because the absolute incidence of mitotic crossing over is small, and estimates of mitotic recombination fractions are unreliable. A comparison of mitotic and meiotic maps made on this basis shows that, although the order of genes is the same, the rate of exchange between them differs during the two processes.

Diploids that have become homozygous at one or more loci but are still heterozygous at others may give rise to homozygotes for the latter by further crossing over in series, as shown in Figure 12.7. Or at any time they may be reduced to the haploid state by *somatic reduction*, a process which brings about recombination among nonhomologous chromosomes without any relation to crossing over. This can be seen from an analysis of the diploid described in Table 12.1. Its composition with regard to four chromosomes was:

Chromosome I	$\frac{su\ ribo\ an}{+ \quad + \quad +} \text{---} \text{O} \text{---} \frac{+ \quad + \quad +}{pro\ paba\ y} \quad bi$
Chromosome II	$\frac{acr\ w}{+ \quad +} \text{---} \text{O} \text{---} \frac{ad_1}{+}$
Chromosome V	$\frac{pyro}{+}$
Chromosome VIII	$\frac{chol}{+}$

Selection yielded 126 yellow *y w*⁺ haploids. These were all *acr*⁺ and, with one exception, *ad*⁺; the *ad* exception is probably a coincidence

of crossing over and haploidization in the same lineage. All of the haploids carried, in unrecombined form, the chromosome with the *y* marker and were, therefore, *su*⁺ *ribo*⁺ *an*⁺ *pro* *paba* *bi*⁺. As a consequence,

TABLE 12.1
Somatic Segregants from an *Aspergillus* Diploid of Constitution
*su-ad*₂₀ *ribo* *an* + + + *ad*₂₀ *bi*
+ + + *pro paba y ad*₂₀ +
(After Pontecorvo, 1958, *Trends in Modern Genetics*,
New York: Columbia University Press)

Selection for Yellow Segregants (<i>y</i> or <i>y/y</i>)															
Haploids						Diploids									
+	+	+	<i>pro</i>	<i>paba</i>	<i>y</i>	<i>su</i>	<i>ribo</i>	<i>an</i>	+	+	<i>y</i>	<i>ad</i> ₂₀	+	35	
					Total	126	+	+	+	<i>pro</i>	<i>paba</i>	<i>y</i>	<i>ad</i> ₂₀		+
						<i>su</i>	<i>ribo</i>	<i>an</i>	+	<i>paba</i>	<i>y</i>	<i>ad</i> ₂₀	+	110	
						+	+	+	<i>pro</i>	<i>paba</i>	<i>y</i>	<i>ad</i> ₂₀	+		
						<i>su</i>	<i>ribo</i>	<i>an</i>	<i>pro</i>	<i>paba</i>	<i>y</i>	<i>ad</i> ₂₀	+	9	
						+	+	+	<i>pro</i>	<i>paba</i>	<i>y</i>	<i>ad</i> ₂₀	+		
Total													154		
Selection for Adenine-Independent Segregants (<i>su</i> or <i>su/su</i>)															
<i>su</i>	<i>ribo</i>	<i>an</i>	+	+	+	<i>su</i>	<i>ribo</i>	<i>an</i>	+	+	+	<i>ad</i> ₂₀	<i>bi</i>	59	
					Total	80	<i>su</i>	+	+	<i>pro</i>	<i>paba</i>	<i>y</i>	<i>ad</i> ₂₀		+
						<i>su</i>	<i>ribo</i>	<i>an</i>	+	+	+	<i>ad</i> ₂₀	<i>bi</i>	21	
						<i>su</i>	<i>ribo</i>	+	<i>pro</i>	<i>paba</i>	<i>y</i>	<i>ad</i> ₂₀	+		
						<i>su</i>	<i>ribo</i>	<i>an</i>	+	+	+	<i>ad</i> ₂₀	<i>bi</i>	181	
						<i>su</i>	<i>ribo</i>	<i>an</i>	<i>pro</i>	<i>paba</i>	<i>y</i>	<i>ad</i> ₂₀	+		
Total													261		

Genetic Map of Chromosome I with Distances Expressed as Fractions of 100 when Total Length of each Arm is Set at that Number							
Mitotic	23	8	69	6	72	22	
	<i>su</i>	<i>ribo</i>	<i>an</i>	<i>o</i>	<i>pro</i>	<i>paba</i>	<i>y</i>
Meiotic	45	23	32	41	20	39	

mitotic crossing over does not seem associated with haploidization. Consideration of the other two chromosomes, for which no selection was made, shows that the segregation of white, nonhomologous chromosomes is random.

Parental Associations			Recombinant Associations		
<i>pyro</i>	<i>chol</i>	24	+	<i>chol</i>	40
+	+	35	<i>pyro</i>	+	27
		59			67

These haploids seem to arise as aneuploids ($2n - a$, where $a = 1$ at the onset of the process, and $a = n$ at the end). At first some chromosomes are in a disomic condition, but their eventual loss may be selected for by the advantage possessed by a fully balanced haploid. Loss is probably achieved by nondisjunction, which would also give rise to the ca. 5 per cent of diploids that are homozygous for all markers in both arms of one chromosome.

The high rate at which chromosome reduction occurs accounts for the fact that *Aspergillus* is not a diploid organism. A rough estimate of the balance of processes can be made as follows:

- 1. Proportion of diploids in a newly synthesized heterokaryon — 10^{-6} .
- 2. Proportion of haploid nuclei produced by diploid nuclei — 10^{-3} .
- 3. Proportion of crossover nuclei produced by diploid nuclei — 10^{-2} .

Without selection, the equilibrium between diploid and haploid nuclei

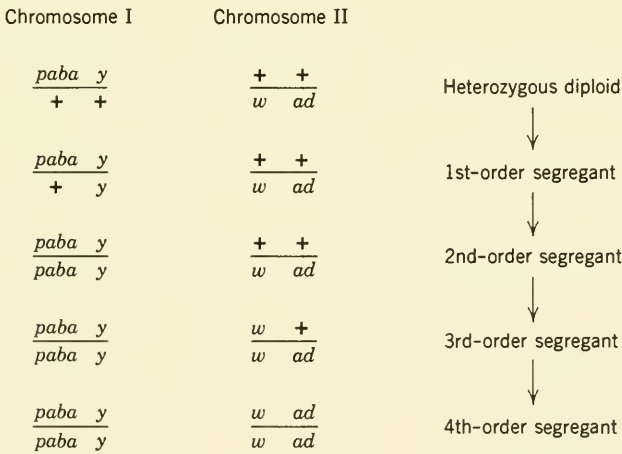


FIGURE 12.7. Sequential development of homozygosity through repeated somatic crossing over.

should be $10^{-6}/10^{-3} = 10^{-3}$, and *Aspergillus* should remain preponderantly haploid despite a persistent tendency of haploid nuclei to fuse. In normally diploid organisms, this balance is otherwise. In vertebrates, at least, there is a strong tendency for diploidization of haploid nuclei. It apparently results from endomitosis, a doubling of the chromosomes without nuclear division. Parthenogenetic haploids that reach maturity inevitably double their chromosome number in the process. The parthenogenetic turkeys at Beltsville, Md. are male because in fowls the male is XX in composition. When sperm from a parthenogenetic male fertilize the X- and Y-bearing eggs of a female, both male and female offspring result. Parthenogenetic frogs, on the other hand, are XX and female.

In some forms, such as *Neurospora crassa*, diploids cannot be formed in heterokaryons composed of nuclei of the same mating type. Presumably this is because of an incompatibility that prevents such nuclei from fusing, even within a fruiting body where karyogamy does occur between nuclei of opposite mating type. In some yeasts, such as *Saccharomyces cerevisiae*, there is normally a diplophase in the life cycle and, during it, somatic recombination is known to occur, stimulated especially by ultraviolet light. This recombination does not involve chromosome reduction to any detectable extent, the tendency for karyogamy throwing the balance in the other direction.

SOMATIC MUTATION AND SELECTION

Diploid organisms frequently undergo changes in chromosome number in the development of some specialized tissues. In the mammalian liver and in the roots of some plants, for example, nuclei form a polyploid series from n through $8n$. These conditions seem to arise at the end of reproductive life and before the cell's specific function is achieved, but their significance is not evident. Nor is it entirely clear what function is achieved by the polyploidy and aneuploidy usually found in tumors and in cells that have been established in tissue culture. Here there is often considerable variation in chromosome number, and the stem-line cells from which the variants are produced have a chromosome complement different from normal. The aberrant chromosome complements usually include chromosome rearrangements and may be the secondary consequences of an adapted condition. The *in vitro* transformation of chicken fibroblasts into *Rous sarcoma* cells by treatment with virus is not at first accompanied by recognizable chromosome abnormalities. But they do later occur, perhaps reflecting the achievement of the

most suitable conditions of the genetic material for multiplication in an environment in which the normal genotype is at a selective disadvantage.

Such selections of favorable genetic constitutions have been demonstrated to take place in fungal heterokaryons where new environments bring about changes in the ratios of pre-existing nuclei, allowing the organism to adapt. But such changes do not provide a useful model for somatic cell variation, except insofar as the vegetative fungal mass may be considered a soma. Heterokaryons are unique; rarely is there more than one nucleus per somatic cell in higher plants and animals. Moreover, in fungi, heterokaryons may be established by the migration of nuclei from other individuals. They also arise by mutation of one of the several contained nuclei. When this occurs in a multinucleate cell such as a bacterium, a heterokaryon is also formed, but the consequence is different, as the number of nuclei is small and the process of nuclear assortment in the daughter cells does not regularly bring together the products of different nuclei. As shown in Figure 12.8, a segregation of the mutant nuclei occurs after a number of generations determined by the number of nuclei per cell. The time of onset of increase in a mutant clone is thereby determined, and the pattern of increase is a function of whether the mutant gene dominates in the heterokaryotic condition. Unicellular forms like bacteria provide better models than fungi for the consequences of mutation and selection in populations of somatic cells. What is known about their behavior has not been con-

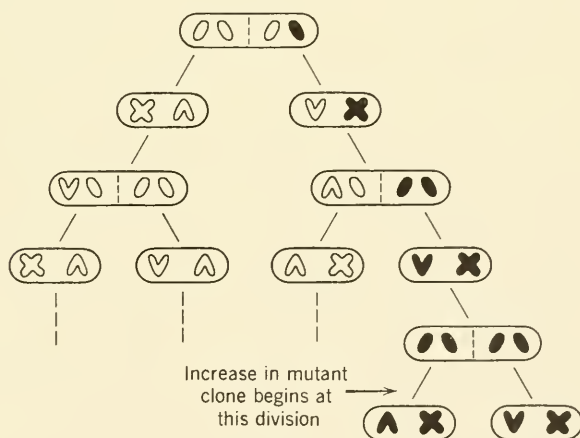


FIGURE 12.8. Diagram of the segregation of a mutant nucleus from its nonmutant sisters in a heterokaryotic bacterial cell (from Ryan and Wainwright, 1954, *J. Gen. Microbiol.*, 11:364).

tradicted by findings in somatic cells themselves. As a matter of fact, experimentation on such cells mimics the approaches undertaken in microbial genetics.

A priori, we could expect that somatic cells must comprise a heterogeneous population because of mutation. If the human body consists of some 10^{14} cells, and the mutation rate for a given gene is as low as 10^{-8} , distributed throughout one individual, there would be considerably more than a million cells carrying a chromosome which was mutant at that locus alone. Aside from technical difficulties inherent in studies of somatic cells, there is the major obstacle of diploidy to overcome. Recessive mutations are not immediately observable in homozygous diploids, but mutations in one of a pair of recessive genes can be detected directly. Many domesticated flowering plants have been selected for their mutability. The bud variegations which modify flower color may include, at least in part, the reproductive cells. Their behavior in crosses shows that the variegation involves a somatic mutation. When variants appear in individuals produced asexually from cuttings or runners, it is difficult to be sure that the change results from mutation at a specific locus because the test of crossing is not available. Similarly, in animals where the soma is usually irreversibly differentiated, somatic variants might arise by other means. But some may occur through mutation.

The inheritance of antigens usually does not involve dominance; each member of a pair of alleles independently causes the production of the one antigen characteristic of it. This is also true of the antigens determining tissue incompatibility. If the antigenic constitution of transplanted tissue is different from that of the host, the transplant, after a sojourn of a variable length of time, will be rejected. The foreign antigen elicits a reaction in the host which eventually destroys cells containing that antigen. This is why successful grafting can usually be achieved only within the same organism or between identical twins. In the adult, those antigens which were naturally present in the cells of the embryo for some reason do not elicit this reaction. Even the injection of foreign antigens into an embryo will protect it as an adult against the same antigen. With experimental animals it is possible by inbreeding to produce essentially isogenic individuals which behave as identical twins in grafting. It is also possible to have lines which are isogenic except that they differ in the alleles at one of the many loci determining histocompatibility.

The Kleins in Sweden have made hybrids between such lines of mice differing only at the *H-2* locus, one of the 14 known to determine histocompatibility. Tumors arising in such mice after treatment with

methylecholanthrene do not grow when transplanted back into the parental lines but do so only when transplanted into the hybrid line. This is because each parent was homozygous for either the $H-2^b$ or the $H-2^s$ allele, while the hybrid was a heterozygote, $H-2^a/H-2^s$. Therefore, the tumor elicited antibodies in the parental hosts which lacked one or the other gene and the corresponding antigen. But sometimes variant cells arise in the tumors, allowing survival in one of the parental hosts; their frequency is increased by X-rays. Serological tests indicated that some of these variant tumors had lost the $H-2^a$ antigen, allowing them to grow in the $H-2^s/H-2^s$ parent (Figure 12.9). In model experiments involving the artificial mixture of compatible and incompatible tumor cells, it was shown that the compatible cells had an absolute selective advantage and grew even when present in proportions as small as 4×10^{-7} , despite the destruction of their incompatible neighbors. As a consequence, variation and selection seem the explanation.

Variation in the constitution of tumors is of common occurrence; usually their antigens are only a sample of those in the cells that produced them. It is not at all sure that point mutations are the basis; chromosomal changes seem usually involved, especially when there is a great increase in the range of transplantability. But even when specific variants arise from controlled heterozygotes, deletions, somatic crossing over, and other mechanisms are *a priori* as probable as point mutation.

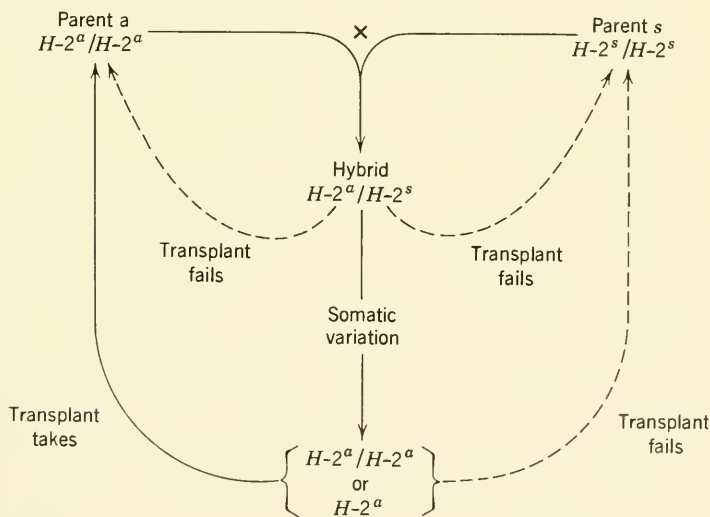


FIGURE 12.9. Diagram of the consequence of somatic variation in a heterozygous tumor.

There is even the possibility that genetic exchange may occur between the tumor cells and those of the host, for the histocompatibility antigens are found in many, if not all, of the cells of the body. There seems to be no tissue specificity in the content of these substances. But, of course, when we have a mosaic, we expect differences as a result of variation not restricted to a given tissue. Such cases are known. For example, the *ABO* antigens detected on the red blood cell surface are usually also secreted in the saliva, but individuals are known in which the antigens in the two sites are different. Furthermore, it appears that everyone is mosaic to a small extent for the antigens of the red blood cells. Although these cells are mixed by the circulation, the blood-forming sites in the bone marrow have small patches of somatic variants.

The demonstration of this fact requires the development of sensitive techniques for the detection of very small frequencies of red cells of aberrant type. One method entails the labeling of a sample of blood cells with radioactive chromate, $\text{Na}_2\text{Cr}^{51}\text{O}_4$, followed by treatment with a specific agglutinin. About 15 per cent of the cells, and hence of the radioactivity, remains in the supernatant. Unlabeled cells of the same type are then added, and agglutination is carried out once more with the result that the radioactivity of the new supernatant is further reduced. This procedure is repeated until the radioactivity in the supernatant reaches a stable level. A knowledge of the dilutions allows calculation of the proportion of nonagglutinable cells. It can be seen from Figure 12.10 that each agglutination step removed the same fraction of marked agglutinable type- A_1 cells and that eventually there was a residue of cells that would not agglutinate. These nonagglutinable cells can be independently isolated and prove to lack the A_1 antigen.

But two types of nonagglutinable cells were found to be present by the use of different specific agglutinins. Type A_1 blood from which the type A_1 cells were removed by A_1 agglutinin showed a further decrease in the proportion of nonagglutinable cells upon the addition of A_2 agglutinin (Figure 12.10). Thus it was shown that type- A_1 blood had not only about 10^{-3} type O cells but about the same frequency of type A_2 as well. Similarly, A_1B blood had both B and A_2B cells in low frequency. The O and B cells would be expected to result from the loss of the gene A_1 , but the A_2 and A_2B cells may involve a replacement of gene A_1 by A_2 . Since neither A_1 nor A_2 is ever found in *cis* arrangement with B , this result could not be obtained by somatic crossing over with an ostensible A_1A_2 cistron. Furthermore, in different blood samples, there is no correlation between the frequency of cells with A_2 antigen and with neither A_1 nor A_2 antigen. Therefore the appearance of the variant cells cannot be explained by any process leading to the

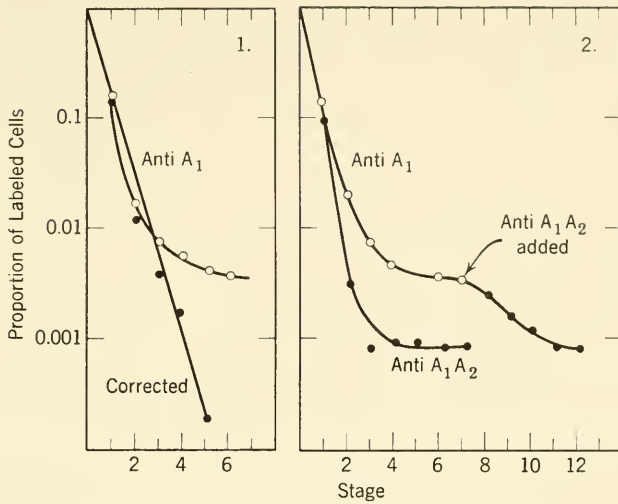


FIGURE 12.10. Isotope dilution curves on cells from an A₁ donor. In (1), the closed circles represent the results of subtracting from the experimental values that value obtained at stage 6. It shows that the agglutinable cells were lost at a constant rate. In (2), the use of two specific agglutinins shows that the A₁ blood had both A₂ and O cells (from Atwood and Scheinberg, 1958, *J. Cell. & Comp. Physiol.*, **52**, Suppl. 97; and Atwood, 1958, *Proc. Natl. Acad. Sci. Wash.*, **44**:1054).

formation of homozygotes. It may be explained as a phenocopy and have no real genetic basis, or it may be a true case of somatic mutation. The bone marrow should behave formally like a chemostat overflowing into the blood stream; with a constant mutation rate and no selection, the number of mutant blood-forming cells would increase with age, perhaps in a linear fashion. It can be calculated that a mutation rate of about 10^{-5} per cell per division would be required to account for a frequency of 10^{-3} mutant cells at age 35. This rate is within the range found in cultured bone marrow cells mutating to drug resistance (Table 2.3). If the rate is calculated per hour, it becomes about 10^{-8} , similar to the rates of mutation of bacteria and viruses. This raises the question of whether the stability of genes in all organisms is about the same, the calculated differences residing in the length of time required to duplicate genes in different organisms.

The process of somatic mutation may be more readily studied in tissue cultures where cells can be grown separately in suspension, plated out to form colonies, and, in general, treated as bacteria. Puck has developed techniques for this purpose which originally involved plating on an irradiated feeder layer of other cells which could not grow but, nonethe-

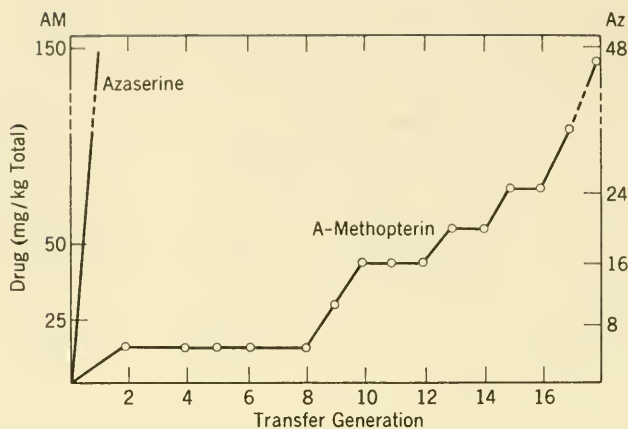


FIGURE 12.11. Selection of resistant forms of mouse tumors in the presence of increasing concentrations of drugs (from Law, 1958, *Ann. N. Y. Acad. Sci.*, 71:976).

less, provided the nutrients required. Later, better defined media containing macromolecular serum fractions and growth factors such as cholesterol and vitamin C were found to support the growth of all the single cells plated. These cells may come from many sources, and upon plating new clones can be established. The chromosome number may be euploid, or, if not euploid after the establishment of the clone, it can be made to remain essentially constant under certain conditions.

Monodispersed cultures of human cells have a genetic stability resembling that of bacteria, but variants do occur despite constancy in chromosome composition. Among them are types with changed nutritional requirement, growth rate, morphology, surface properties, and drug and virus sensitivity. For example, a change from a type consisting of polygonal cells in a compact colony to fusiform types forming a loose colony occurs at a rate of ca. 8×10^{-5} per cell per division. The fusiform type, unlike the polygonal type, is refractory to infection by polio virus and can be selected without exposure to the virus because of its different morphology. This shows the spontaneous origin of this trait. In another case, the fluctuation test of Luria and Delbrück was employed to show that resistance to A-methopterin by leukemia tumor cells was not induced by the drug (Table 12.2). Variation and selection brought about the change. The same seems true of the transformation of solid tumors into ascites cells which grow freely in the fluid of the body cavities. In one experiment when an average of 4.3 cells producing solid tumors were implanted, 7 out of 8 transfer lines gave rise to ascites

cells. Variation and selection in the development of drug resistance in tumors may take different paths; just as in bacteria, high-level resistance may appear in one step or after several (Figure 12.11). But the one-step resistant cells are sometimes readily reversible in the absence of the drug, pointing up once again the possibility that mechanisms other than gene mutation are responsible.

One alternative to mutation in chromosomal genes is mutation or loss of some cytoplasmic factor. Not much is known about such processes in somatic cells, but there is precedent to suppose that they do take place (see Chapter 9). In the corn plant, for example, plastid mutations occur in the presence of the chromosomal gene *iojap*, and sometimes mutations seem to occur spontaneously. In higher plants and in the cells of microorganisms, plastids may be modified or lost in the presence of chemicals such as streptomycin, or as a consequence of other environ-

TABLE 12.2

**Variance Analysis of the Mean Weight of Leukemic Tumors
Developed in the Presence of Antitumor Agent A-Methopterin, from Sublines
Maintained Independently for 7 Transfers and for Subdivisions
Made from a Single Line at the Last Transfer**
(from Law, 1952, *Nature*, 169:628)

Independent Sublines				Single Line			
Subline Number	Number of Growths		Mean Weight, mg	Subdivision Number	Number of Growths		Mean Weight, mg
	+	-			+	-	
1	9	1	181.9	13-I	6	4	22.0
2	3	7	8.5	II	5	5	67.7
3	3	7	8.0	III	6	4	51.0
4	10	0	99.3	IV	8	2	54.5
5	6	4	69.2	V	6	4	58.0
6	8	2	30.3	VI	6	4	36.2
7	7	3	31.0	VII	6	3	41.4
8	0	10	0.0	VIII	8	2	47.4
9	3	7	7.5	IX	8	2	27.0
10	9	1	88.3	X	8	2	42.0
11	9	1	199.0		$\chi^2 = 5.29$, mean = 44.8		
12	0	10	0.0		$P = 0.80$, $\sigma^2 = 195.9$		
14	4	5	74.8				
15	7	3	68.1				
$\chi^2 = 61.85$, mean = 61.85							
$P = 0.001$, $\sigma^2 = 4144.84$							

mental stimuli such as the absence of light. Although plastids are a special cell structure, others behave similarly. The kinetosomes at the bases of flagella seem to have autonomy in reproduction and the chance of their loss may be increased by the presence of drugs such as acridine dyes. Some forms, such as *Paramecium bursaria*, *Chlorhydra viridissima*, and *Convoluta roscoffensis* (a flatworm), have endosymbionts, green algae which live in the cytoplasm to the mutual benefit of both organisms. They are passed from cell to cell at division and, in the last two forms, to the egg at ovulation. The symbionts behave as cell particulates and may be lost upon cultivation in certain environments. These and many other cases provide models for the possible behavior of similar entities known to occur in the somatic cells of higher organisms.

INFECTIOUS HEREDITY

Recombination mechanisms that do not involve sex provide another means whereby somatic cells may vary. Although claims are beginning to be made, no sure evidence has been presented for transformation or for fusion and exchange between cells in tissue culture. Nor has clear evidence for genetic exchange come from a study of chimaeras in plants or animals where the soma consists of cells from different individuals. In plant graft-hybrids, cells of different genotype may exist side by side, as some layers of cells come from the scion and some from the stock. Similarly, when animal tissues are grafted, there is opportunity for genetic exchange between cells. Claims have been made that this does occur. When a pigmented patch of skin is transplanted into a white area of the same mammal, pigmentation spreads beyond the margins of the graft. These newly pigmented areas can be propagated. The cytoplasmic extensions of the pigment-forming cells in the skin can be observed to fuse with others, and are thought to permit the spread of pigment-forming particles from one cell to another. The possibility that the spread of pigmentation is due to the migration of the pigment-forming cells themselves has not been rigorously excluded, however.

Viruses provide a vehicle for the transfer of genetic material from cell to cell. The study of animal viruses has been facilitated by the use of tissue-culture techniques; when spread on sheets of fibroblast cells, the viruses form plaques which can be counted and classified morphologically. From these plaques pure lines may be established of many viruses, such as those causing poliomyelitis, influenza, and encephalitis. Their growth within single cells in monodispersed cultures can also be examined, and in all regards they are similar to bacteriophage, except

that they reproduce more slowly and sometimes do not lyse the host cell upon their release. There are even semistable associations made in tissue culture between polio virus and resistant cells in which the cells may be killed as they yield new virus, or they may multiply, carrying the virus forward into future generations. In some ways this condition resembles that of lysogenic bacteria; many other provocative examples exist among plants and animals.

In mice there is the classic example of the mammary tumor virus. When transmitted at coitus or via the milk at nursing, it may induce a tumor, depending on the genetic susceptibility of the host and the endocrinological stress experienced. It may propagate in males and genetically resistant females without giving rise to cancer. Sometimes this virus arises spontaneously in stocks thought to be free of it, raising the question whether it may be of genetic origin in the same sense as is a prophage. Another example is Shope's papilloma virus which can be extracted from warts on the skin of the wild American cottontail rabbit; when applied to the scarified skin of the same kind of rabbit, it produces new papillomas and more virus. But in the domestic rabbit of European origin it gives rise to tumors which become cancerous with age. In these tumors the virus causing them cannot be demonstrated, although particles with similar antigens can be found. If the virus is injected into cottontails in which papillomata have been induced by tar, cancerous growths result. In these again the virus is not found, but virus antigen is present. X-ray treatment causes the virus to be produced. This virus seems to be able to enter a provirus stage, which in many ways resembles that assumed by prophage. Many other cases of masked viruses exist; more and more tumors are being found to be associated with viruses and, as tissues from the body are cultured, more and more are found to produce viruses with time. The main factor limiting our ability to discover this kind of association is the requirement for a tester system in each case. It is beginning to appear that most cells contain proviruses and that they may be normal features of the genotype.

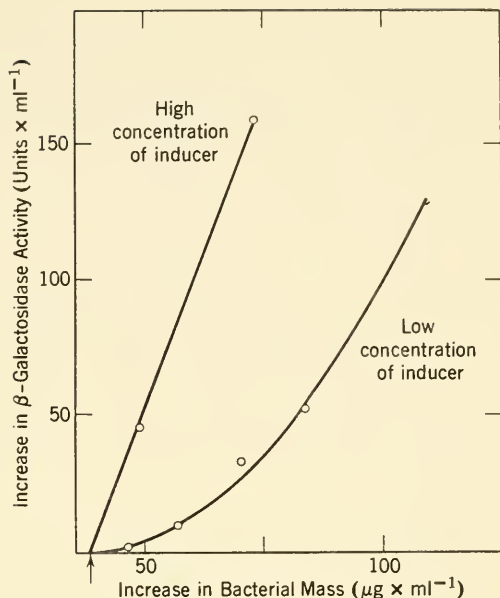
In *Drosophila* there are cases of infection with viruslike entities. One causes a pathological sensitivity to high concentrations of CO_2 . It is not contagious but is infective by organ transplantation or by the injection of body fluids from one fly to another. After injection there is an eclipse period of about one day, whereupon the virus multiplies and spreads throughout the body. Before it has been stabilized, females transmit it to some of their offspring, but males transmit it to none; after stabilization, females transmit it to all, and males to some of their offspring, as though its concentration had increased. This pattern is one of maternal inheritance. Indeed, the virus is transmitted to offspring independently

of chromosomal markers and probably has an extrachromosomal residence. This supposition is supported by the fact that flies infected with virus may be cured by heat shock, some only temporarily. The virus, *sigma* (σ), may mutate into several other types. But the most interesting condition is an association, *rho* (ρ), resembling that in lysogenic bacteria, because infected flies are resistant to CO_2 until a certain age when they become sensitive. In the interim they are immune to infection and do not yield virus. When the viruses are extracted from aged ρ flies, they are found to be indistinguishable from σ taken from other sources. All this is a strong indication of a provirus state, and the transformation into infectious virus upon senescence has implications for the origin of diseases of age. Instances of transduction and lysogenic conversion have yet to be reported. The fact that many animal and plant viruses have RNA as genetic material rather than DNA raises interesting questions in this regard.

PHENOCOPIES

A final category of mechanisms by which somatic cells may vary is that of phenocopies. A *phenocopy* is a phenotype modification which mimics some mutant condition but is not inherited at sexual reproduction. Exposure of *Drosophila* larvae to high temperatures will cause some to develop mutant wings or lozenge eyes, but not because of a change in genotype. Nonetheless, the change does have a somatic hereditary basis, the cells of the soma reproducing the same modification. A fine study of this kind of hereditary mechanism was made by Novick on the induced synthesis of β -galactosidase in *E. coli*. When an inducer is present in saturating concentrations, a linear relation is found between the appearance of new β -galactosidase and the synthesis of new protein (Figure 12.12). This suggests that all cells participate uniformly in the synthetic process, a concept confirmed by other lines of experimentation. It is otherwise when the concentration of inducer is low; there is then a lag before enzyme synthesis begins at an exponential rate, eventually reaching that observed with high levels of inducer. The accelerating phase of enzyme formation is ascribable to a heterogeneity in the population; only a fraction of the cells become capable of being induced at one time; they transmit this ability to their progeny. The other cells cannot do this. As may be recalled from Chapter 11, in order for the inducer to enter the cell, a transport enzyme, or permease, must be formed. Once inside the cell, the inducer causes the formation, not only of β -galactosidase but also of more permease, leading to the transport of more inducer, and so

FIGURE 12.12. Formation of β -galactosidase in *E. coli* in response to different concentrations of inducer (from Cohn, in: McElroy and Glass (eds.), *The Chemical Basis of Development*, Baltimore, The Johns Hopkins Press, p. 458).



on, in an autocatalytic fashion. At low levels of inducer, every cell has a small constant probability of forming a molecule of permease; once it does, the process of induction goes exponentially to completion. The process of enzyme induction is under these conditions an all-or-none process resembling mutation, and is transmitted cytoplasmically to the daughter cells as long as the inducer is present in the environment. This is a good example of an autocatalytic mechanism determining a hereditary difference between cells and independent of any change in chromosomal genes or extrachromosomal particles other than the enzymes themselves.

It seems likely that mechanisms involving reversible expression of phenotype are the best candidates for the origin of the orderly variation in cell type that leads to normal development. It is conceivable that almost all cells respond to a stimulus from the environment through a nonchromosomal mechanism. Although the possibility must be left open, it seems less likely that cells could behave in such a uniform way through chromosomal mechanisms which may rather give rise to pathological processes, initiated nonuniformly in single cells. It should not be assumed, however, that those types of variation that are random in their origin need give rise to a disorderly result. The necessary order can be achieved by the cellular environment; population change may be

directed by conditions which allow the multiplication of only certain kinds of cells. Although selection of variant cells may not be the most important factor in normal development, when variants do arise, selection may follow. The quality and intensity of the change will be a function of the nature of the variant and the nature of the environment.

CELL POPULATION DYNAMICS

Some information is available about the explosive changes which occur when microorganisms are exposed to drastically inhospitable environments such as those containing toxic agents or lacking required growth factors. The various cells making up the population may also interact with one another as, for example, in the modification of the environment to make it favorable or unfavorable for one or another type. The selective actions involve the length of the lag before division, the rate of multiplication, the efficiency with which the limiting factor is utilized, and the rates of death during or after growth. Were it not for selection and certain features of the expression of the variant phenotype, for every allele an equilibrium should eventually be established with all others to and from which it can mutate. The equilibrium would be achieved when the proportion equals:

$$\frac{\text{Number of mutants}}{\text{Number of parents}} = \frac{a}{b} \quad (12.1)$$

where a is the rate of change to the variant, and b to the parental condition. As a consequence, considering the large number of genes behaving in this way, the population should gradually become so heterogeneous that it would not consist of predominantly one cell type. In practice, this kind of heterogeneity is not observed. Bacterial cultures of one cell type do not degrade into such a mixture despite the fact that their haploid nature exposes mutations.

The reason for this contradiction is that while a particular mutant is rising in frequency in a population, other kinds of mutations are also occurring. Even a constant environment presents a challenge to a growing population; types fitter than their relatives will be selected for and overgrow all others, including the mutant whose rise was being observed. The result is that these mutants will be eliminated, only to start their march toward equilibrium again among the newly established fitter types. As this process continues, a new kind of equilibrium results (Figure 12.13). Its level is determined by the rates of mutation and the degree of fitness achieved rather than only by the rates of mutation

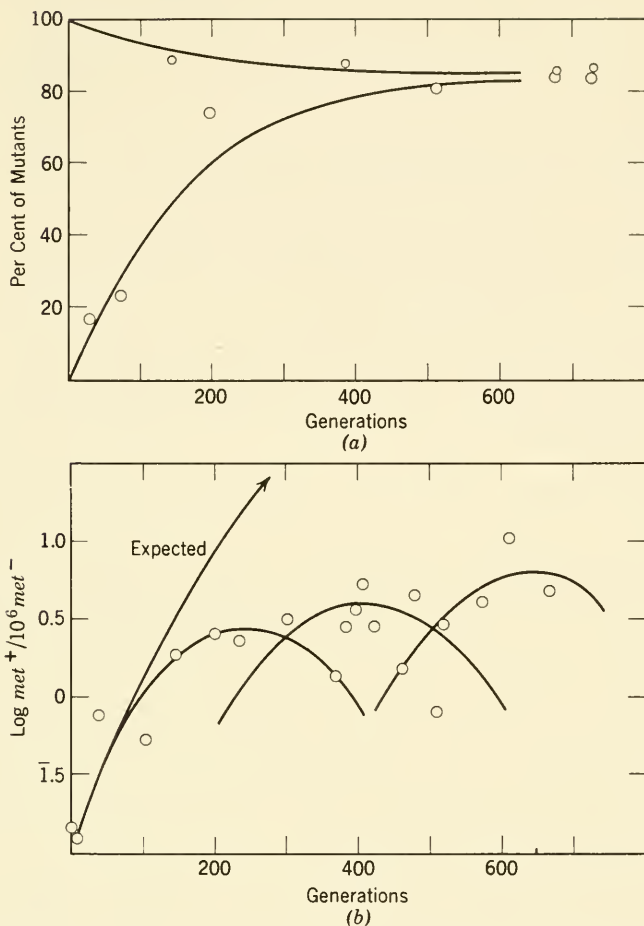


FIGURE 12.13. Mutational equilibrium and orthoselection in populations of bacterial cells.

(a). Achievement of mutational equilibrium for antigenic mutants in *Salmonella* where the rates of mutation for these characters are unusually high. (b). Operation of orthoselection in *E. coli* in maintaining the level of methionine-independent mutants in a methionine-requiring population at a level of ca. 10^{-6} instead of 10^{-2} which is the ratio of mutation rates ($10^{-8}:10^{-6}$) (from Stocker, 1949, *J. Hygiene*, 47:398; and Atwood, Schneider, and Ryan 1951, *C. S. H. Symp. Quant. Biol.*, 16: 345).

to and from the allele whose equilibrium is under consideration. This process is called periodic selection or *orthoselection*. By means of it, bacterial populations maintain a certain degree of uniformity. At the same time they constantly improve themselves for the existing environment and contain a small proportion of mutants which might be preadapted for some future environment. The principle involves a continued selection of subclones within the population and may well operate for any kind of variants among diploid somatic cells.

The problem of selection is a large and complex one about which little is known as yet. But it should be clear that population dynamics, while certainly part of the subject of ecology, are at the same time a genetic phenomenon. Population dynamics are presently being considered as a means by which the formation of antibodies may be understood. It had previously been supposed that these specific globulins in the blood-forming tissues of the body were synthesized on the model of the introduced antigen. Alternatively, it now seems possible that the plasma cell population may be genetically heterogeneous with regard to the antibodies it can produce. This heterogeneity may be achieved through spontaneous mutation as the plasma cell tissue arises in development. Selection is thought to operate in two ways. First, immature plasma cells may be *repressed* by antigens reacting with the antibodies they produce; hence, in the adult there are no cells capable of producing antibodies against the animal's own antigens (tissues such as the lens of the eye, which are out of contact with the circulatory system, being an exception). Second, in the adult, a foreign antigen may electively *stimulate* the multiplication of a clone of cells already endowed with the capacity to synthesize the corresponding antibody; the persistence of this clone would account for continued immunity.

Experiments have been performed to determine whether different plasma cells can produce only one or another antibody. Single plasma cells from animals immunized against two antigens (flagella of *Salmonella* or bacteriophages) were found to produce only one or the other, or to produce both antibodies, according to the condition of immunization. Although exclusiveness is clearly not complete, it is still possible that the plasma cells may have restricted potencies.

In any event, it should be clear that there are quite a number of ways in which hereditary differences may be established among somatic cells. In all too few cases can we yet be sure which, if any, are operating. There is little doubt, however, that as studies on this subject continue, especially when modeled on the approaches found fruitful with populations of microorganisms whose component cells are a soma, we may expect that there will be found facts and principles of some theoretical

interest and of considerable practical consequence for fields such as pathology and embryology.

BIBLIOGRAPHY

- Allen, G. (ed.), 1960. A symposium on cytology and cell culture genetics in man. *Am. J. Human Genetics*, **12**:97-138 (especially the contributions of Chu, Ford, Barr, Sachs and Fraccaro).
- Biology Division, Oak Ridge National Laboratory, Tenn., 1958. Symposium on genetic approaches to somatic cell variation. *J. Cell. Comp. Physiol.*, **52**, Suppl. 1:1-409.
- Biology Division, Oak Ridge National Laboratory, Tenn., 1960. Symposium on mammalian genetics and reproduction. *J. Cell. Comp. Physiol.*, **56**, Suppl. 1:1-193. (Especially paper by R. Owen.)
- Hannah-Alava, A., 1960. Genetic mosaics. *Sci. Amer.*, May, pp. 119-130.
- Pontecorvo, G., 1958. *Trends in Genetic Analysis*. 145 pp. New York, Columbia University Press.
- Strong, L. C. (ed.), 1958. Genetic concept for the origin of cancer. *Ann. N. Y. Acad. Sci.*, **71**:807-1241.
- Wolstenholme, G. E. W., 1959. Biochemistry of human genetics. *Ciba Foundation Symposium*. 347 pp. Boston, Little, Brown & Co. (especially the panel discussion on tissue culture, pp. 278-303).

13

The Summing Up

In writing this book we have tried to include within each chapter not only a summary of the current knowledge, presented in terms of illustrative experiments, but also a discussion of areas of uncertainty and unsolved problems. In any developing science, one is confronted with the array of past achievements which have molded the science as it is known today, and simultaneously with the areas of ignorance which still exist. It is not easy to present a balanced view. Often, in textbooks, problem areas are consigned primarily to the last chapter. Since we have considered unsolved problems throughout the book, our purpose in this last chapter is of a different nature. We shall attempt a very brief general view of the present state of cellular genetics, in respect to the science itself and to its impact upon certain other areas of learning and of everyday life.

At the outset, we proposed to consider cellular heredity in terms of the nature of hereditary materials, the analysis of transmission mechanisms, and of the translation systems by means of which control is exerted over metabolism and development. It seems appropriate, then, to summarize the book with reference to these categories.

NATURE OF HEREDITARY MATERIALS

Nucleic acids have been directly identified as carriers of genetic information in bacterial and viral systems, and a great diversity of indirect experiments support the hypothesis that nucleic acids serve this function in all organisms. Most of the available information concerns DNA, which is the active principal in bacterial transformation. The demon-

stration of *in vitro* DNA synthesis by replication from pre-existing DNA provides the chemical basis for the genetic autonomy of this class of molecules.

We have come to associate heredity with particular macromolecular configurations. As a general definition, we have proposed that heredity consists of the conservation of specificity during replication, and, in this sense, a hereditary determinant must replicate with conservation of specificity. The possibility that other chemical constituents besides DNA carry genetic information has not been excluded. The genetic material of some viruses is RNA, but the chemistry of RNA replication is not known. If there are structural elements of the cell, such as membranes or organelles, which contain genetic information, it may not be possible to study them by classical genetic analysis or by transfer from cell to cell; in that event, the demonstration that they replicate would provide evidence of genetic autonomy.

Thus, the stage is being reached at which chemical and biological tests may be used not only to supplement each other but also as alternative methods for the demonstration of the same point.

The identification of genes has depended upon their ability to mutate. Mutant genes have provided the material for genetic analysis, and studies of the mutation process have provided an avenue of approach to the identification of genetic material. For example, the treatment of RNA and DNA with nitrous acid *in vitro*, followed by biological assay of the treated nucleic acids (by infection and transformation respectively), has shown that the deamination of a single nitrogen base may result in mutation. In attempts to decipher the genetic code, the known specific action of particular mutagens will be of great value.

What classes of hereditary factors have been overlooked because they do not mutate? Nonchromosomal genes almost fall into this category. As yet, no systematic method has been developed for the recovery of nonchromosomal mutants, although the use of acridine dyes and streptomycin appears promising. All sorts of phenotypic traits have been found to be under genetic control, but the methodology of genetics provides no assurance that most of the determinants of cell heredity have been recognized.

The great advances of the last few years make possible the rephrasing of problems open to experimental attack in this area of genetics: the detailed analysis of gene chemistry and of the mutation process, the systematic search for new classes of hereditary determinants, and the application of mutation analysis to the study of gene replication. For biochemists there is the further challenge to discover replicating molecules other than DNA.

TRANSMISSION MECHANISMS

The linear arrangement of genetic material has been established as a characteristic feature of organisms from bacteriophage to man. Presumably this linearity is a consequence of the geometry of the nucleic acid molecule, which is a linear polymer. In classical genetic theory, genes were viewed as indivisible units strung along a one-dimensional chain. With the development of methods for the analysis of recombination within a gene, the evidence of linearity has been extended virtually to the nucleotide level. In all organisms from phage to man, the chromosomal gene consists of a functional unit responsible for the appearance of a particular gene product, and composed of a linear array of hundreds or even of thousands of nucleotides. Presumably genetic information resides in the linear sequence of these nucleotides which is rigorously maintained in replication.

The transmission of these giant polymers to progeny cells without loss of their linear order has been achieved by means of chromosomes. In the bacteria and viruses, mapping has thus far indicated an invariant order which does not differ from strain to strain. In *E. coli*, for example, despite the evidence of chromosome breakage by insertion of the *F* factor, no evidence has been obtained of any inversions. Furthermore, the gene order in the related genus, *Salmonella*, seems to be the same as in *E. coli*. In organisms with true chromosomes in the sense of complex nucleohistone bodies, considerable rearrangement of the gene order has occurred. Translocations and inversions are common in wild populations. The significance of these differences will probably remain obscure until more is known of the organization of the genetic material.

The most perplexing question in the field of transmission genetics concerns the mechanism of recombination between linked genes. Whether exchanges occur during replication (Belling's hypothesis) or during the prophase pairing of preformed chromosomes (partial chiasmatype hypothesis), or whether both processes occur (or something quite different from either one) remains one of the immediate and challenging questions in genetic analysis today. It is related of course to other unknowns: Is DNA present as a continuous double helix or are there non-DNA connecting links? Are there functional differences of significance between the bacterial chromosome and that of higher organisms? What controls chromosome breakage and rejoining, and the healing of broken ends? What is the structural basis of the phenomenon of position effect?

The study of chromosome rearrangements by conventional recombination analysis led to the recognition of position effect in the 1930's. Two

apparently different classes of position effect were described: the S-type and the V-type. The S-type, in which two pseudo-allelic mutations produce only mutant phenotypes when located on separate chromosomes (*trans*), but the wild-type phenotype, when their $+$ -alleles are recombined on the same chromosome (*cis*), is now called the *cis-trans* position effect. This phenomenon, as we have seen in Chapter 6, is now interpreted as the requirement for continuity of a functional unit consisting of hundreds of nucleotides.

The V-type position effect, however, originally recognized as the influence of a neighboring region upon the expression of a particular gene is not understood at all. It is only now becoming possible to reopen the investigation of this phenomenon in which both chromosomal structure and gene function are intimately involved. In fact, the dichotomy between structure and function, so useful in the early development of genetics, is now being replaced by a more integrated approach, essential for a more penetrating analysis of genetic mechanisms. A main limitation in the development of an integrated view has been in the area of gene action.

GENE ACTION

No over-all picture of gene action can be synthesized from the existing evidence with any sense of assurance. Nonetheless, it may be worthwhile to reorganize the material presented in Chapters 9, 10, and 11 in a more unified form. We shall attempt to do this by considering the various elements of a working genetic system which have been described, together with some proposals about their modes of interaction.

At the chromosome level, three kinds of determinants have been described. They are the functional genes, which we shall refer to collectively as *S*, the controlling elements, *O*, located adjacent to *S*, active only in the *cis* position, and able to repress the expression of wild-type *A*, and another set of controlling elements, *R*, which may be located anywhere in the genome, and which act by activating *O*. In addition to these elements, there may also be nonchromosomal genetic factors involved in the action of every gene. All these determinants have been identified operationally by their phenotypic effects. How do they correspond to chemically defined components?

It seems a reasonable extrapolation from known data that the chromosomal components are DNA, but even here there is some doubt; perhaps some are non-DNA constituents. The difficulty in detecting a regularly associated protein in the bacterial chromosome tends to exclude protein

from consideration for this role. Chromosomal RNA is a possible candidate, since it has been reported as a constituent of the chromosomes of higher forms, although there is no evidence for or against its presence on the bacterial chromosome. However, it is a pure speculation that RNA may be involved in gene expression as a mapable part of the chromosome.

With respect to the nonchromosomal genetic factors, most likely they are not DNA since no trace of nonchromosomal DNA has been detected in most cell types. Many classes of RNA molecules surely exist, in addition to those already recognized, and some of them may carry primary genetic information. In addition to the pathway of protein synthesis described in Chapter 11, other pathways probably exist, for example, concerned with the synthesis of insoluble, membrane-bound proteins. Which molecules carry the code for those proteins?

A somewhat less speculative question concerns the possible genetic role of RNA molecules which are known to be involved in protein synthesis: transfer RNA and microsomal RNA. The transfer RNA presumably carries specificity for combining with specific amino acid activating enzymes. Whether that specificity is transmitted by replication from pre-existing RNA or by synthesis from DNA is not known.

The ribosomal RNA is a candidate for the carrier of some primary genetic information. Since proteins are synthesized on the ribosomes, it has been widely postulated that ribosomal RNA carries the genetic code by means of which the amino acids are polymerized in proper sequence. If this is correct, then the question becomes one of establishing the origin of the ribosomal RNA. Is it replicated and therefore genetic, or is it synthesized on a DNA template? From existing information obtained in *Neurospora* and in higher forms, it seems likely that ribosomal RNA originates in the nucleus, but is it of chromosomal or of nucleolar origin?

Two principal possibilities have been considered: (1) that ribosomal RNA is replicated in the nucleolus and, consequently, carries primary genetic information; or (2) that it is synthesized on a DNA template, and is a gene product, carrying the DNA code into the cytoplasm where proteins are synthesized. Although many investigators favor the second alternative, there is little direct support as yet beyond the fact of amino acid substitution in mutant hemoglobins.

Alternatively, the ribosomal RNA could be the product of the combined action of a chromosomal gene of class S, giving rise to an RNA gene product, and a genetically autonomous RNA replicating in the nucleolus. On this hypothesis, part of the primary genetic code for protein specificity would reside in the RNA and part in the DNA; ribo-

somal multiplication would be controlled by the part which comes from the chromosome. Although a dual information system is postulated, the interaction of the two components within the nucleus greatly minimizes the problem of "searching out and finding." If the RNA component is present in many replicates, the chance of random mutation of many of them at once is vanishingly small. The chromosomal system would provide the flexibility for evolution by mutation.

Now let us see how these components might interact. Following the discussion of Chapter 11, we consider a system in which the ability of *S*, the class of functional genes, to act is controlled by *O*, the class of linked controlling elements. The activation of *O* in turn is determined by a gene product from *R*, the class of unlinked controlling elements. Since we expect that normally these systems are regulatory in nature, a feedback signal from the cytoplasm is required to complete the circuit.

How does the feedback operate? This question is equivalent to the more conventionally phrased query: How does the cytoplasm influence gene expression? Studies of enzyme formation have provided evidence that the repressor-inducer ratio may represent the governing cytoplasmic state which is ultimately responsible for regulating the rate of enzyme formation in wild-type cells. So far as is known, repressors and inducers are chemically related small molecules, metabolites, which can compete for particular sites.

Another class of compounds which have been identified by their action are those which carry a signal from controlling elements *R* to *O*. On the simplest scheme, the activation of *R* would be determined by the cytoplasm (i.e., by the repressor-inducer ratio), and *R* would activate *O* with no further regulation from the cytoplasm. Since *O* and *R* may be far apart on the genome, however, it seems likely that the signal from *R* to *O* goes through the cytoplasm, and this may provide a second point of cytoplasmic influence upon the course of gene action. Finally, enzyme formation itself occurs in the cytoplasm, and it is likely that some cytoplasmic control may be exerted at that point.

It has been demonstrated in a striking way, both in bacteria and in maize, that controlling elements act by *repression* of wild type gene expression. Thus activation of *R-O* represses *S*, while inactivity of *R-O* allows wild-type expression of *S* to occur. This is a new, unexpected, and extremely important finding in the analysis of gene action. It makes possible the formulation of much more precise questions for experimental attack than have been posed before. For example, one may begin to pinpoint the site of repressor action, and thereby of cytoplasmic control of gene expression.

It now appears that the analysis of gene action requires two kinds of information: the identification of all the components of the system, and the recognition of which element is blocking phenotypic expression at a particular time. Since repressor action mimics mutation, it is essential to find a way to distinguish between them. On our present view, repression should be much more readily reversible than mutation, and in microbial systems at least, an operational distinction should be possible. In higher organisms, however, or indeed even in *Paramecium*, where growing conditions are kept very uniform, the reversibility of cytoplasmically determined repression may be extremely difficult to demonstrate. This difficulty may limit the usefulness of such organisms for these aspects of genetic analysis.

The distinction between repression and mutation may also be a key problem in the analysis of differentiation. In the nuclear transfer experiments, for example, changes in some genetic constituents are detected by transfer of nuclei from late stages of larval development back into very early stages. These changes exhibit surprising stability in subsequent subcultures carried out by transfer of nuclei back into very young blastulae after allowing 12 to 14 divisions at each step. In these experiments, the genetic changes exhibit a permanence which seems to exclude repression of the sort we have been considering. Nonetheless, differentiation as a general process involves the restriction of potentialities, and in this sense, it may involve the "turning off" of some genetic elements. Because of the regularity of timing and the 100 per cent effectiveness of the turning-off process, investigators have been loath to invoke the concept of mutation. Repression, which is a directed, highly specific, and highly efficient process, provides a more acceptable conceptual basis for an orderly restriction of potentialities than does mutation. Where does the permanence come from? Perhaps the conversion of the conditional permanence of cytoplasmically induced repression to a more stabilized permanence, resembling that of mutation, is at the root of differentiation.

REPLICATION AND THE ORIGIN OF LIFE

The scientific analysis of evolution has been one of the fruitful areas of inquiry stemming from the development of genetics. Conventionally, the study of evolution was restricted to considerations of organisms as they are known from natural history and from paleontology. In recent years, a particularly exciting new field of research has appeared, inquiring into the origin of life itself. Deriving much of their impetus from

the pioneering formulations of the Russian chemist, Oparin, scientists from many disciplines have begun to do experiments designed to test specific hypotheses concerning the origin of life.

Oparin proposed that life originated on earth at a time when the seas were rich nutrient soups full of complex organic molecules. He developed a detailed scheme for the chemical evolution of more and more complex compounds, beginning with the gases thought to be present in the atmosphere when the earth cooled down—methane, ammonia, water, and hydrogen. Subsequently, following a different line of reasoning, Urey came to similar conclusions about the reducing atmosphere of the earth at this time, and proposed that the formation of organic compounds might be catalyzed by ultraviolet light and by electric discharges in storms. In the absence of free oxygen, there would have been no screening of the high-energy short-wave-length ultraviolet radiation from the earth by the ozone layer of the atmosphere, as there is now.

The first experimental demonstration of the origin of simple amino acids and other organic molecules under conditions similar to those postulated by Urey was carried out by circulating hot water in an atmosphere of hydrogen, methane, and ammonia, with energy provided by a spark discharge. The products, separated by chromatography and characterized chemically, included several straight-chain amino acids and other organic acids, as well as carbon monoxide, carbon dioxide, and nitrogen in the gaseous phase. Recently adenine too has been formed in such a system. Attempts to synthesize other aromatic compounds such as pyrroles and pyrimidines, have not yet succeeded, but it seems likely that with the proper choice of conditions, including the presence of trace metals and surface catalysts, ring compounds characteristic of biological material will be formed. These experiments, and similar ones performed subsequently in other laboratories, have established the principle that organic compounds can arise in the absence of life under conditions fairly similar to those existing while the earth was cooling.

Although the huge organic solution existing then would tend toward disorder, the direction may have been reversed, rarely and locally. For example, the adsorption of materials on clay surfaces is thought to have accounted for the presence of only one of the optically active isomers of many compounds, and at just such sites the necessary initial organization may have been conferred upon the organic world by the pattern of inorganic templates. The fact that these early reactions occur in the absence of oxygen is of great importance. If oxygen had been present in the atmosphere preceding the origin of life, there would have been two disastrous consequences: the compounds being formed would have been oxidized and lost; and an ozone layer would have accumulated in the

atmosphere, screening the short-wave ultraviolet radiation from the earth. The experimental results are in accord with a second hypothesis: that most of the molecular oxygen in the atmosphere is of biological origin, more specifically, of photosynthetic origin.

In this anaerobic oceanic broth, given the millions of years needed for improbable events to occur, molecules may have come together spontaneously to form aggregates of different types with varying degrees of order. Perhaps natural selection played a role at this early time, so that those aggregates better able to take up substances from their environment and capable of overcoming the problems of size by duplicating would win out in the competition for materials. Furthermore, some complexities could be virtues. When the supply of certain substances became low, those aggregates which assumed the capacity of synthesizing the limiting factor from other substances would have a selective advantage. The ability to undergo anaerobic fermentation and secure energy and substrates by breaking down organic molecules would give the aggregate a degree of freedom from its environment and confer a selective advantage. Thus primitive "organisms" may have been established, with gradually increasing organization, structure and complexity, from which we inherit those features of cells so widely distributed as the common denominators of living things.

As long as no organisms were about, chemical evolution could continue. But with the appearance of the first living things, the organic chemical environment would be consumed, metabolized, and changed. Organisms evolved, making their own organic substances from those inorganic materials in essentially limitless supply, such as carbon dioxide, which is constantly being formed as a product of fermentation. Photosynthesis is one way in which organisms learned to make sugar from carbon dioxide and water. The catalyst which traps the energy in sunlight is the pigment, chlorophyll. Some of the precursors from which it is synthesized today are able to absorb light and may have been catalysts in the past. They would have been improved upon step by step as the ability to make the complex chlorophyll molecule evolved.

The earth's atmosphere gradually became pervaded with molecular oxygen, a by-product of photosynthesis. Now aerobic respiratory metabolism could be evolved as an efficient improvement over anaerobic fermentation. It may be envisaged that the supply of compounds required by these primitive systems underwent localized depletion. If so, any form capable of carrying out a terminal step in biosynthesis would have enjoyed a great selective advantage, since presumably the penultimate compound would still have been present in excess. Horowitz developed a scheme of this sort, by which biosynthetic pathways may

slowly have evolved—backwards. Once organisms could synthesize their substance from inorganic compounds, life was no longer dependent upon the primordial organic broth, and food cycles became possible.

Eventually the conversion of oxygen into ozone would have formed a layer high in the atmosphere which is nearly impervious to the heavy ultraviolet radiation from the sun. In this and many other ways conditions favorable for the origin of life disappeared. It seems unlikely that life is repeatedly arising spontaneously on this planet nowadays; but even if it were, it would be almost immediately destroyed by existing organisms. Whether life has independently arisen elsewhere in the universe is a fascinating question which may be answered now in terms of faith or speculation, but which really requires more information than we have at present. We need especially to know about the possibility of alternative forms of life.

Now let us inquire cautiously into that explosive question: What is life? Physiologists interested in the origin of life have emphasized the requirements of energy availability and structural organization, and geneticists have stressed the requirement that the system must replicate. It seems evident that both criteria are essential. At the symposium held in Moscow in 1957, "The Origin of Life on the Earth," many aspects of current research and speculation were presented; the reader is referred to the proceedings, published in English, for details.

An essential feature of the first living unit was its ability to multiply by a copying process, giving rise to more of the same units. If nucleic acids are the only kinds of molecules able to replicate, then it follows that nucleic acids must have been an essential constituent of the first living unit. Pushing this reasoning to its logical extreme, Horowitz has proposed that a molecule of nucleic acid was the first living thing on earth.

The weak point of this argument is the assumption that only nucleic acids can replicate. The problem which we have encountered repeatedly in this book, the recognition of hereditary determinants other than chromosomal genes, involves the same unknowns. If replicating cell constituents other than nucleic acids, such as the supertemplates discussed in Chapter 9, are found, then clearly they must be considered in relation to the origin of life. Even if it is shown that only nucleic acids replicate in organisms living today, the question must still be investigated whether some simpler replicating system might have existed under the conditions of early life.

In the 1920's genes were described as units capable of autocatalysis and heterocatalysis. Today we speak of *replication*, *decoding*, and *regulation*. In terms of evolution, we consider it likely that replication

was achieved long before living things had acquired built-in regulatory devices. It is illuminating that the controlling elements of gene regulation which are now being discovered act by repression of otherwise functioning genes. Perhaps in the earliest living forms, all the genetic material functioned all the time, an awkward and wasteful situation, with a tremendous selective advantage accruing to any system which could control this outpouring. We may even consider the possibility that the primitive replicating units were cytoplasmic structures and protein-forming sites, and that today some genetic information still resides in these materials.

Superimposed upon this rather chaotic blob of protoplasm, a very orderly regulatory system was evolved, characterized by its own internal organization, a controlled time and manner of replication, and a virtually infinite capacity to evolve by mutation and selection—in a word, the chromosomes. At this point, we may leave the alluring but shadowy realm of biopoiesis and uncontrolled growth and approach the more solid ground of genetic science upon which has been built the experimental analysis of the evolution of life as we know it today.

BIBLIOGRAPHY

- Horowitz, N. H., 1945. On the evolution of biochemical syntheses. *Proc. Natl. Acad. Sci. Wash.*, **31**:153-157.
- Lederberg, J., 1960. Exobiology: approaches to life beyond the earth. *Science*, **132**:393-400.
- Oparin, A. I., 1953. *The Origin of Life* (2nd ed.). New York, Dover Publications.
- Oparin, A. I. (ed.), 1959. *International Symposium on the Origin of Life on the Earth*. London, Pergamon Press.
- Schrödinger, E., 1945. *What is Life?* London, Cambridge University Press.
- Tax, S. (ed.), 1960. *Evolution After Darwin*. Vol. 1. *The Evolution of Life*. 620 pp. Chicago, The University of Chicago Press.

Postscript

Progress in the field of molecular genetics has been so sweeping in the interval since this book was written, that with the reprinting of the first edition, we feel that some account of recent advances, however brief, is mandatory. This postscript is limited to an extension of the discussion in Chapters 11 and 13 on mechanisms of gene action and will deal specifically with (1) messenger RNA, (2) protein synthesis and the genetic code, and (3) the relation of specific amino acid alterations in particular proteins to the corresponding nucleotide changes in DNA. It is hoped that this account will aid the reader in assimilating the new findings which are revolutionizing both concepts and experimental methods in biology.

I. MESSENGER RNA

Several independent lines of evidence have led to the formulation of the messenger RNA hypothesis, which postulates the existence of a class of RNA molecules, complementary to DNA, which carry genetic information from DNA to the ribosomes where they control protein synthesis. In 1956 Volkin and Astrachan detected, in phage-infected *E. coli*, the synthesis of an RNA fraction with a base composition mimicking that of the phage DNA. Subsequently, an analogous RNA fraction whose base composition mimicked that of the DNA of the same strain, was detected in uninfected cultures of several bacteria. In the phage-infected system, Brenner, Jacob and Meselson showed in 1961 that an RNA fraction, newly synthesized after phage infection, could be found attached to pre-existing ribosomes. Taken together, these observations support the messenger hypothesis.

In 1960, independently in three laboratories (Hurwitz *et al.*, Weiss *et al.*, Stevens), an enzyme called RNA polymerase was described which catalyzes the DNA-dependent synthesis of a complementary RNA. The base composition of the newly synthesized RNA is rigorously determined by the DNA used as primer, in a manner wholly analogous to the replication of DNA catalyzed by DNA polymerase (p. 186). The four ribonucleoside triphosphates are required for RNA synthesis and the polymerization leads to the release of stoichiometric amounts of pyrophosphate. The DNA acts as a primer or catalyst in the sense that the RNA product formed greatly exceeds the input DNA in quantity; but DNA is also the template since the RNA formed is complementary to it.

There is as yet no proof that the RNA polymerase system functions in the cell to make complementary RNA, but the existing evidence supports this view. Further indirect evidence comes from the demonstration by Hall and Spiegelman in 1961 of the presence in phage-infected and in uninfected bacteria of DNA-RNA hybrid molecules, presumed to have been isolated while in the process of RNA synthesis on the DNA template. They also showed that artificial hybrid molecules could be formed from DNA and a special RNA fraction; the hybrid-forming ability was attributed to a close complementarity in base sequence between the DNA and RNA. Hybridization was proposed as a criterion for identification of a complementary RNA of biological origin corresponding to the *in vitro* product of RNA polymerase activity.

A method has been developed by Bautz and Hall for the isolation of T4 messenger RNA corresponding to a particular genetic segment, using the mutant phage T4 carrying a deletion in the *r II* region (p. 169). The method involves formation of DNA-RNA hybrid molecules between T4-DNA and the RNA formed after infection of *E. coli* with T4. Messenger RNA corresponding to the deleted region of DNA will be formed when wild-type T4 is used but not when the mutant strain is used for infection. Formation of hybrid molecules, in this experiment, occurs on a chromatographic column to which DNA is adsorbed first, and RNA then added. When both DNA and RNA come from the wild-type system, the complementary RNA of the deleted region is adsorbed onto the column. However, when the DNA comes from the mutant strain, and the RNA from cells infected with the wild-type, then the RNA molecules corresponding to the deleted region are not hybridized on the column, and are thereby separated from the rest of the RNA. The fact that this experiment works is in itself a substantial confirmation of the existence of messenger RNA, as well as providing a powerful new analytical tool.

II. PROTEIN SYNTHESIS AND THE GENETIC CODE.

The genetic coding problem was first formulated by George Gamow who proposed a triplet code in which the translation of genetic information from the four-letter nucleotide alphabet to the 20 letter amino acid alphabet would require a minimum coding ratio of three nucleotides to one amino acid. Within the past year this proposal has not only been tested experimentally, but an actual translation of the code has been achieved. "Breaking the code" was made technically feasible by the development of a reliable *in vitro* system in which the incorporation of amino acids into polypeptides could be studied.

The system developed by Nirenberg and Matthaei with cell fractions from *E. coli* involves the known constituents: ribosomes, cell supernatant containing the amino acid activating enzymes, the s-RNA's, ATP and an ATP-generating system, and the 20 amino acids for the continuing synthesis of the 20 amino acid-s-RNA complexes. When such a system is incubated with radioactive amino acids, a measurable incorporation of label into protein occurs. Nirenberg found that the rate and extent of incorporation could be greatly increased by adding any one of a variety of phenol-extracted RNA preparations. These results led him to test the response of the system to synthetic polynucleotides and in so doing he established a method for deciphering the genetic code. He found that the addition to the system of polyuridylic acid (poly U) led to a great increase in uptake of label, resulting from the incorporation of only one of the 20 amino acids, phenylalanine, into a polyphenylalanine peptide. Thus it was shown that uridylic acid alone coded for phenylalanine. A direct estimate of the coding ratio was not possible because poly U acted catalytically. Subsequently, purely genetic studies by a group at Cambridge have provided other evidence that a triplet is sufficient.

Subsequently, with the use of synthetic polynucleotide copolymers, a set of triplets for all 20 amino acids have been worked out in the laboratories of Nirenberg and of Ochoa. As an example, with the copolymer poly UG, containing about five times as many uridylic acid residues as guanylic, several amino acids are incorporated, including phenylalanine, leucine, valine, glycine, and tryptophane. From the relative amount of each of these found in the protein fraction, it is possible to distinguish two classes, relative to phenylalanine: leucine and valine, in which the incorporation is about 1/5 that of phenylalanine, and glycine and tryptophane in which the incorporation is very much lower. On this evidence it has been proposed that the coding letters for leucine and valine are

UUG, and for glycine and tryptophane are UGG. Similarly, code letters have been inferred for the other amino acids, using other copolymers: UA, UC, UAC, UAG, and UCG. It is noteworthy that the code is degenerate in the sense that more than one triplet has been found to code for leucine and for a few other amino acids. Since there are 20 amino acids and 64 triplets, some degeneracy was expected. Not all of the possibilities have yet been found. It may require a use of other organisms and perhaps a different method to complete the translation. Until that is accomplished, the universality of the code will remain an open question.

The correctness of the code as presently known is supported by the remarkable consistency with which it fits the data on known amino acid substitutions in proteins resulting from mutation. For example, the amino acid composition of the tobacco mosaic virus (TMV) protein has been established, and single amino acid changes have been found by Wittman and by Tsugita and Fraenkel-Conrat in a series of mutant viruses resulting from *in vitro* deamination by HNO_2 (p. 37). In 16 of these mutants, the amino acid changes when coded in terms of the corresponding triplets, could be accounted for by deamination of a single nucleotide of the triplet. There are also a few instances which do not fit the present code, and may reflect its degeneracy. All of the known amino acid substitutions in mutant hemoglobins have also been examined, and most of the changes here too are consistent with single nucleotide changes in the nucleic acid code.

Support of the universality of the code comes from *in vitro* studies of protein synthesis in mixed systems with various components coming from different organisms. Perhaps the most dramatic example is the synthesis of TMV protein by the *E. coli*-ribosome-supernatant system to which TMV-RNA was added as "messenger" or template-RNA. This result also represents the first reported instance of the *in vitro* synthesis of a protein of known properties determined by an added template-RNA.

Although the amino acid sequence of newly synthesized protein is determined by m-RNA, there is an obligate requirement for s-RNA, and it is responsible for the actual translation step. Although m-RNA carries the code, one may ask: does it "know" the code? To answer this question, a direct experiment was designed and performed in the laboratories of Lipmann and of Benzer. The cysteine-s-RNA complex was isolated and, by reaction with Raney nickel, the cysteine was converted to alanine, giving rise to a new complex carrying the amino acid alanine but the code for cysteine. This complex was then introduced into an *in vitro* system for protein synthesis. Alanine was incorporated in the place of cysteine, demonstrating that the specificity of the s-RNA and not that of the amino acid itself or of the m-RNA determined the incorporation.

III. CORRELATION BETWEEN AMINO ACID SUBSTITUTIONS AND GENE MUTATIONS.

Work with several bacterial proteins as well as with hemoglobin and TMV-protein has all supported the hypothesis that single nucleotide changes in the gene can result in single amino acid substitutions in the corresponding protein. Although single nucleotide changes are not the only molecular basis of mutation, they clearly represent one important class.

In the tryptophane synthetase system of *E. coli* (p. 276), amino acid substitutions have been identified in some mutants. For example, in mutant A-23, glycine present in the wild-type has been changed to arginine in one position in the protein. In the mutant A-46, which maps at the same site, the same glycine has been changed to glutamic acid. According to the present code, glycine is coded by UGG, glutamic acid by UAG, and arginine by UCG. Thus the change from glycine to glutamic acid involves the single nucleotide change from G to A, and in the case of arginine from G to C.

No biochemical method exists as yet for determining the coding sequence within the triplet, but Yanofsky has presented a genetic method for achieving this result. Although mutants A-23 and A-46 map at or near the same site, it is possible to recover rare wild-type recombinants. If the mechanism of recombination is actually at the nucleotide level, then the recovery of the wild-type makes possible an ordering of the triplets. Glutamic acid would have to be coded UAG and arginine UCG, in order to recover glycine which is UGG. The other recombinant, predicted but not found, would have to be UAC and would provide further confirmation. Of course the direction in which these triplets are read is not yet known.

It is appropriate to conclude this summary of recent advances in molecular genetics with an experiment that epitomizes the nature of this field, combining the most elaborate biochemical methods of fingerprinting and amino acid sequence analysis with the sophisticated simplicity of the genetic cross.

BIBLIOGRAPHY

- Cold Spring Harbor Symp. Quant. Biol. **26**:1-408 (1961) Cellular Regulatory Mechanisms.
- Bautz, E. K. F. and B. D. Hall, 1962. The Isolation of T4-specific RNA on a DNA-Cellulose column. Proc. Natl. Acad. Sci. Wash. **48**:400-408.

- Crick, F. H. C., Barnett, L., Brenner, S. and R. J. Watts-Tobin, 1961. General nature of the genetic code for proteins. *Nature* **192**:1227-1232.
- Nirenberg, M. W., and J. H. Matthaei, 1961. The dependence of cell-free protein synthesis in *E. coli* upon naturally occurring or synthetic polynucleotides. *Proc. Natl. Acad. Sci. Wash.* **47**:1588-1602.
- Henning, U. and C. Yanofsky, 1962. An alteration in the primary structure of a protein predicted on the basis of genetic recombination data. *Proc. Natl. Acad. Sci. Wash.* **48**:183-190.
- Speyer, J. F., Lengyel, P., Basilio C., and S. Ochoa, 1962. Synthetic polynucleotides and the amino acid code, IV. *Proc. Natl. Acad. Sci. Wash.* **48**:441-445.

Author Index

- Adams, M. H., 149
 Allfrey, V. G., 354
 Ames, B., 330
 Anderson, E. G., 252
 Atwood, K. C., 301, 303, 375, 353
 Auerbach, Ch., 39, 59
 Avery, O. T., 13, 15, 32
- Barratt, R. W., 90
 Bateson, W., 64
 Baur, E., 235
 Beadle, G. W., 29, 30, 32, 33, 90, 92, 112, 173, 273, 274, 361, 362
 Beermann, W., 320, 354
 Belling, J., 77, 110, 111, 112
 Benzer, S., 164, 165, 174
 Beyerinck, M. W., 274
 Bonner, D. M., 303
 Boveri, T., 357
 Breuer, M. F., 320
 Brehme, K. S., 90
 Bridges, C. B., 90, 91, 92, 93, 101, 339
 Briggs, R., 344, 351, 353
 Brink, R. A., 344, 354
 Britten, R., 309
 Burnet, F. M., 149
- Case, M., 196, 199, 206, 292, 303
 Catcheside, D. G., 90, 149, 269, 303
 Cavalli-Sforza, L. L., 59
 Chargaff, E., 33, 206
 Chase, M., 33
- Cohen, G. N., 354
 Cohen, S., 115
 Cohn, M., 324, 332, 354, 355, 351
 Cooper, K. W., 109
 Corey, R. B., 13, 255
 Correns, C., 234, 255, 270
 Coulson, C. A., 46-49, 59, 116
 Creighton, H. B., 107, 105, 109, 112
 Crick, F. H. C., 10, 33, 115, 121, 164, 217
- Davidson, J. N., 33, 206
 de Harven E., plate X
 Delbrück, M., 44, 46, 59, 376
 Demerec, M., 47, 149, 155, 159, 160, 174, 220, 362
 DeVries, H., 219
 Dobzhansky, Th., 51, 104
 Doermann, A. H., 119
 Doty, P., 299
 Dubinin, N. P., 340
- Emerson, R. A., 344
 Ephrussi, B., 240, 255, 256, 270, 361
 Ephrussi-Taylor, H., 132, 149
- Farquhar, M. G., plates XII, XIII
 Fincham, J. R. S., 304
 Fling, M., 33
 Fraenkel-Conrat, H., 24, 33
 Franklin, N., 170
 Freese, E., 165, 169, 170, 204, 205, 216, 232
 Frenster, J. H., 354

- Gall, J. G., 182, 206
 Garnjobst, L., 90
 Garrod, A. E., 274
 Garry, B., 330
 Gierer, A., 38
 Giles, N. H., 196, 199, 206, 223, 225, 232, 292, 293, 303
 Glover, S. W., 149, 174
 Goldschmidt, R., 354
 Gorini, L., 329, 354
 Gots, J. S., 149, 174
 Green, M. M., 196
 Griffith, F., 12, 14
 Grunberg-Manago, M., 312
 Grüneberg, H., 272, 273
- Hall, C. E., plates III, IV
 Hämmerling, J., 305, 306, 307, 354
 Hannah-Alava, A., 385
 Hartman, P. E., 149, 161, 174
 Hartman, Z., 149, 174
 Hawirko, R., 45
 Hayes, W., 136, 149
 Hershey, A., 23, 33, 120
 Hogness, D. S., 354
 Hollaender, A., 224, 232
 Hopkins, J. W., 354
 Horowitz, N. H., 31, 33, 301, 302, 304, 394, 396
 Hotchkiss, R. D., 17, 19, 20, 33
 Hottinguer, H., 240, 256
 Hughes, W. H., 192
 Hunt, J. A., 281
- Iino, T., 347, 355
 Ingram, V., 281, 282
 Itano, H. A., 295
- Jacob, F., 136, 137, 141, 143, 144, 145, 147, 148, 149, 337
 Janssens, F. A., 107
 Johanssen, W., 2, 151
 Jones, D. F., 259
- Kaufmann, B. P., plate VIII
 Kellenberger, E., 205, plate II
 Kendrew, J. C., 289
 King, T. J., 351, 353, 354
 Kiritani, K., 230
 Klein, G., 372
 Kornberg, A., 186, 188, 206, 311
- Kozloff, L., 22
- Laughnan, J. R., 346
 Law, L. W., 376, 377
 Lea, D. E., 46-49, 59, 116, 232, 347
 Lederberg, E., 52, 59
 Lederberg, J., 52, 59, 127, 135, 139, 140, 149, 355, 396
 Leupold, V., 304
 Levinthal, C., 116, 117
 Lewis, E. B., 153, 196, 355
 Linderström-Lang, K. U., 286, 304
 Loper, J. C., 161
 Luria, S. E., 44, 45, 46, 49, 59, 149, 376
 Lwoff, A., 123, 149, 246, 270
- Maas, W., 329, 354
 MacLeod, C. M., 15, 16, 32
 MacLeod, H. L., 33
 Mangelsdorf, P. C., 112
 Marcou, D., 261
 Marmur, J., 299
 McCarty, M., 15, 16, 32
 McClintock, B., 93, 96, 98, 107, 108, 109, 112, 218, 341, 346, 348, 355
 McLeish, J., 90
 McMaster, R., 185
 McQuillen, K., 309
 Mendel, G., 28, 34, 60
 Meselson, M., 141, 187, 188, 191, 206
 Meves, F., 235
 Mirsky, A. E., 179, 180, 354
 Mitchell, H. K., 33, 239, 255, 275, 304, 362
 Mitchell, M. B., 201, 206, 239
 Monod, J., 322, 324, 325, 326, 333, 337, 354, 355
 Moore, S., 286
 Morgan, D. M., plate V
 Morgan, T. H., 34, 66, 91, 234, 270
 Mukai, F., 303
 Muller, H. J., 39, 59, 103, 104, 302, 340
 Mundry, K. W., 38
- Nakada, D., 211, 228
 Newcombe, H. B., 45
 Newmeyer, D., 90
 Norman, A., 222
 Novick, A., 202, 209, 212, 226, 233, 380
- Ochoa, S., 312
 Olive, L. S., 200, 201

- Oparin, A. I., 393, 396
 Owen, R. D., 90
 Ozeki, H., 149, 174
- Palade, G. E., plates I, IX, XI, XII, XIII, XIV, XV, XVI
 Pardee, A. B., 337, 355
 Pauling, L., 13, 284, 285
 Pavan, C., 319, 320, 355
 Perkins, D. D., 90
 Perutz, M. F., 288, 289, 304
 Pollock, M. R., 322, 355
 Pontecorvo, G., 154, 172, 174, 362, 368, 385
 Porter, K. R., 270
 Pritchard, R. M., 204, 366
 Puck, T., 375
 Punnett, R. C., 64
- Renner, O., 235
 Rhoades, M. M., 57, 112, Plate V, VI, VII, 235, 250, 251, 258, 270, 345, 346, 355
 Richards, F. M., 287
 Riley, H. P., 223
 Ris, H., 179, 180, 206
 Rizet, G., 261
 Roberts, R. B., 309
 Roger, M., 21
 Roman, H., 240
 Roper, J. H., 366
 Rotman, R., 120
 Rudner, R., 211
 Ryan, F. J., 46, 211, 230, 371, 383
- Sager, R., 90, 237, plates IX, XV, XVI
 Sams, F., 220
 Sanaia, Q. X., 319
 Sanger, F., 280, 304
 Schaeffer, P., 131
 Schecroun, F., 261
 Scheinberg, S. L., 375
 Schellman, J. A., 304
 Schneider, L. K., 383
 Schramm, G., 24
 Schrödinger, E., 396
 Schuster, H., 59
 Serman, D., 161
 Singer, B. A., 33
 Singer, S. J., 295, 304
 Slayter, R. O., Plate IV
 Slonimski, P., 256, 270
 Smith, L. F., 304
- Snoad, B., 90
 Sonneborn, T. M., 262, 270, 348, 355
 Spackman, D. H., 286
 Spemann, H., 358
 Spiegelman, S., 322
 Srb, A. M., 90
 Stadler, L. J., 39, 173, 219
 Stahl, F., 141, 187, 188, 191, 206
 Stanley, W. M., 149
 Stein, W. D., 286
 Steinberg, D., 290
 Stern, C., 90, 107, 108, 109, 112, 350, 359
 Stocker, B. A. D., 129, 383
 St. Lawrence, P., 304
 Streisinger, G., 170
 Strelzoff, E., 211
 Stubbe, H., 210
 Sturtevant, A. H., 92, 112, 199, 339, 340, 361
 Sueoka, N., 33, 299
 Sunakawa, S., 227
 Sutton, E., 341
 Swanson, C. P., 90, 110, 112
 Szilard, L., 207, 209, 212, 226, 355
- Tatum, E. L., 29–31, 149, 273, 274
 Taylor, J. H., 185, 192, 194, 205, 206, 315, 316, 317
 Timofeeff-Ressovsky, N. W., 39, 59
 Tomizawa, F. I., 227
- Uetake, M., 125
 Urey, H. C., 393
- Vaughan, M., 290
 Vogel, H. J., 355
 Von Wettstein, V., 235
- Waddington, C. H., 355
 Wainwright, L. K., 371
 Wagner, R. P., 33, 255, 275, 304, 362
 Watson, J. D., 10, 33, 118, 121, 164, 217
 Weinstein, A., 111
 Weissmann, A., 357, 358
 Westergaard, M., 231
 Wettstein, D., 270
 Wexler, I. B., 87
 Wiener, A. S., 87
 Wilkins, M. H. F., 33, 179
 Williams, R. C., 33
 Winge, Ø., 235
 Witkin, E. M., 141, 210, 228, 233

- Wollman, E., 136, 137, 141, 143, 144, 145,
147, 148, 149
Woods, P., 192, 317
Woodward, V., 293
Yanofsky, C., 279, 304
Yura, T., 149, 174
Zalokar, M., 314, 355
Zamecnik, P. C., 308, 355
Zelle, M., 224
Zinder, N. D., 127, 149

Subject Index

- Aberrant tetrads, 197–202
Abortive transduction, 129–130, 162
Acetabularia, 305–307, 358
Acridine dyes, 268–269
 effect on *F* factor, 143, 146
 euflavin and yeast, 255–256
 proflavin as a mutagen, 216, 232
 tryptoflavin, 246
Action spectrum, photoreactivation, 225
 UV and mutation, 224
Activator (Ac), 342–346
Active site, 290, 331
ad-4 locus, 292–297
Adenine, 5–12
 mutagen, 212–213
 RNA synthesis, 317–318
Adenine synthesis, 154–157, 171
Adenosine, 5
 antimutagen, 212–213
Adenylic acid, 5, 7
Adenylosuccinase, 168, 277, 292–297
Aegilops, 105
Alkylating agent, 217, 231–232
Allele, 31
 allelic complementation, 291
 cis-trans test, 162
 definitions, 40, 62, 151
 multiple, 171
 segregation in *Sordaria*, 200–201
 transformation, 20
Alpha helix, 284–285
Altered enzymes, 274–280
 Altered enzymes, in suppressed mutants, 298
Amino acid sequence, 280
 cytochrome *c*, 290
 folding, 284–291, 289–290
 hemoglobin, 281
 insulin, 285
 ribonuclease, 285–287
2-Aminopurine, 169, 212, 216
Amorph, 99
Amphidiploid, 104–105
Anaphase, 68–71, plates VI and VII
Anaphase bridge, 103
Aneuploidy, 106, 369, 370
Antibody formation, 384
Antigen, *ABO*, 374–375
 blood, 374–375
 enzymes, 276
 in *Paramecium*, 348–350
 MN, 85–87
 tissue incompatibility, 372–374
Antigenic variation, blood groups, 374–375
 Paramecium, 348–350
 Salmonella, 347–348
 tumors, 372–374
Antimutagen, 212–213, 216
Arginine synthesis, 29–31, 327–329
Ascaris, 357
Ascospore, *Neurospora*, 67
 Sordaria, 200–201
Ascus, in *Neurospora*, 67–69
 in *Sordaria*, 200–201
Asexual reproduction, 2, 71–73

- Asexual spores, 68-70
Aspergillus nidulans, 154-155, 167
 life cycle, 363
 nucleotides per map unit, 172
 somatic crossing over, 362-370
 somatic reduction, 367-370
 Autogamy, 263-266
 Autoradiograph, *see* Radioautography
 Auxotroph, 29-30, 274
- B chromosomes, 95
Bacillus cereus, penicillinase, 322, 326
 subtilis, recombination, 148
 Bacteriophage, *see* Phage
 Balbiani ring, 320
 Bar eye, 99, 339-340
 Barrage, 260-261
 Base analogue, 169, 212-217
 Base ratios, 9
 Beta-galactosidase, 290, 323-327
 genetic control, 334-338
 permeases, 331-334
 phenocopy, 380-381
 Biopoiesis, 396
 Biotin synthesis in *Aspergillus*, 155-158
 Bivalent, 70, 107
 Blepharoplast (basal bodies or centrosomes), 246
 Blood group inheritance, 85-87
 Breakage-fusion-bridge-cycle, 97-98
 5-Bromouracil, 169, 213-214, 216
- C¹⁴, 311
 Cancer, 106, 376-377, 379
 Centriole, 242, plates IX, X
 Centromere, 68-73, 94-95
 Chemical evolution, 394
 Chemostat, 207-209, 212-213, 328, 375
 Chi square (χ^2), 63
 Chiasma, 107
 interference, 79, 109-111
 Chiasmotype theory, 109, 202-206
Chlamydomonas, DNA replication, 189
 life cycle, cytology, 60-61,
 plates IX, XV, and XVI
 streptomycin resistance, 236-238, 259
 Chloramphenicol, bacterial mutation, 210,
 227-229
 DNA synthesis, 226-228
 phage infection, 115
 repressor formation, 338
 Chloramphenicol, RNA synthesis, 313
 Chlorophyll, 253-254
 Chloroplast, 248
 Chlamydomonas, 242, plates XV, XVI
 disc, 248
 Euglena, 232
 grana, 248
 somatic mutation, 377-378
 Chromatid, 68-71, 106-107
 half, 183
 interference, 109-111
 parental, 74
 recombination, 74
 Chromocenter, 95
 Chromomere, 80, 94, 181-182
 Chromosome, Ac, 342-346
 asynchronous synthesis, 195
 Balbiani rings, 318-320
 breakage and rearrangement, 96-104,
 217-219, 222-223, 341-346
 chemical composition, 178-180
 Chlamydomonas, plate IX
 DNA, 25, 178-180
 Drosophila salivaries, plate VIII
 Ds, 341-346
 duplication, 184-195
 E. coli, 143-147
 genes, 66-78
 histone, 27, 179-180
 meiosis, 68-73, plates V, VI, VII
 microscopic structure, 180-183
 morphology, 93-95
 nucleic acid synthesis, 316-321
 phage, 122, 137-149
 polyploidy, 104-106
 puffs, 318-320
 recombination models, 202-206
 RNA, 27, 180
 site of DNA synthesis, 312
 somatic crossing over, 359-370
 somatic reduction, 367-370
 supernumerary, 357
cis-arrangement, 153-158
cis-trans test, 162, 167-168, 291, 340
 Cistron, 167-171, 291
 CLB method, 103-104, 302
 Clone, 3, 44, 351-353
 CO₂ sensitivity, 264-268, 379-380
 Coding, 8, 32, 299-300, 310
 Colchicine, 193
 Complementarity, in DNA, 10

- Complementarity, in s-RNA, 194, 217-218
complementation, *Aspergillus*, 167-168
 in vitro, 295-298
 map, 291-293
 mechanism, 294-297
 Neurospora, 291-297
 Salmonella, 162
 yeast heterocytosomes, 257
Conjugation, bacteria, 135
 Paramecium, 262-266
Constitutive mutants, 334-338
Controlling elements, *Ac*, 342-346
 action, 350
 Ds, 341-346
 Dt, 56-57, 345-347
 H, 348
 o gene, 334-338
 Spm, 346-347
Copy choice mechanism, bacterial recombination, 142
 models, 121, 202-206
 Neurospora recombination, 198-202
 phage recombination, 121
 transduction, 128-129
 transformation, 132
Copy-error, 207, 209-217
Corn, *see* Maize
Cristae, 247
Crossing over, 2, 79
 absence of in phage, 120-121
 cytological evidence, 107-109
 diploids, 89
 interference, 109-111
 inversion heterozygote, 102-103
 models, 202-206
 multiple, 75-77, 79, 106-112
 physiological influences, 111-112
 sister-strand, 109-111
 somatic, 359-370
 suppressor, 103-104
 unequal, 339
Cross-reacting material (CRM), 278-279, 297-298
Cryptic mutant, 332-333
Cytidine, 5
 tritiated, in RNA synthesis, 315-316
Cytidylic acid, 5, 7
Cytochrome enzymes, 240-241, 246-247, 254-257
 active site, 290
 cytochrome *c* synthesis, 311
Cytoplasmic inheritance, *see* Nonchromosomal inheritance
Cytosine, 5-13

Deficiency, *see* Deletion
Deletion, 57, 98-99, 172
 phage, 164-167
 Salmonella, 162
 X-ray induced, 217-218, 222-223
Density gradient centrifugation, 188-191
 DNA, 299-300
 transducing phages, 128
Deoxyadenosine, 5
Deoxycytidine, 5
Deoxyguanosine, 5
Deoxyriboadenylic acid, 5-6
Deoxyribocytidylic acid, 5-6
Deoxyriboguanylic acid, 5-6
Deoxyribonucleic acid, *see* DNA
Deoxyribose, 5-6
Deoxyribothymidylic acid, 5-6
Deoxythymidine, 5
Dicentric chromosome, 96-98
Differentiation, 305-307, 345, 351-354, 356-383, 392
Diphtheria bacillus, 124
Diploid, 68-73
 Aspergillus, 363-364
 DNA, 178-179
 genetics, 83-90
Dissociator (Ds), 341-346
DNA, 4-12
 amount per gene, 166-167
 and mutation, 213-217, 225-229
 base ratios, 9
 base ratios and gene action, 299-300
 chromosomes, 25, 176-177
 control of synthesis, 312
 DNA content of cells, 178
 double helix, 10-12
 E. coli, 183-184
 fibers, plate III
 genetic analysis, 173
 genetic material, 15-17
 in vitro synthesis, 186-189, 311-312
 kappa, 264
 kinetoplast, 246
 melting out temperatures, 21-22
 molecular weight, 191
 phage infection, 114-118
 phages, 22-24

- DNA, reaction with nitrous acid, 36-39
 replication, 116-118, 177, 186-188
 replication and recombination, 202-206
 synthesis and protein, 225-229
 timing of synthesis, 184-186
 transduction, 125-131
 transformation, 15-22, 131-134, 141-142
- Dominant, 86-89
 in *E. coli*, 136
- Dotted gene (Dt), 56-57, 345-347
- Double helix of DNA, 10-11, 118
- Drosophila*, Bar eye, 99, 339-340
 ClB method, 103-104, 302
 CO₂ sensitivity, 264-268, 379-380
 cytological demonstration of crossing over, 107-109
 evolutionary role of inversions, 104
 eye color, 39, 361-362
 gene distribution on chromosome, 303
 generation time, 35
 genetic map, 80-81
 lethal, 362
 mutation, 218, 220
 no crossing over in male, 111
 nondisjunction, 91-93
 nucleotides per map unit, 172
 position effects, 338ff
 position pseudoallelism, 153, 340
 RNA synthesis, 317-321
 salivary chromosome, 80-81, 303, plate VIII
 sex-linked inheritance, 91-93
 sex ratio, 264-268
 somatic crossing over, 359-362
 visible vs. lethal mutants, 271, 302
- Drug resistance, A-methopterin, 21, 376-377
 micrococin, 21
 penicillin, 17-22
 streptomycin, 17-22
 sulfonamide, 21
 tissue culture, 376-377
- Duplication, 98-99, 162, 172, 339
- Duplication process, chromosomes, 184-195
- Electron transport particles, 247
- Endomitosis, 106, 370
- Endoplasmic reticulum (ER), 245
 Chlamydomonas, plate XVI
 kidney, rat, plate XII
 liver, rat, plate I
- Enzymes, altered, 274-280
- Enzymes, antigens, 278
 components, 278
 β -galactosidase, 290, 323-327
 gene control, 30-31, 273-276
 induced synthesis, 321ff
 metal sensitivity, 297-298
 penicillinase, 322, 323, 326
 regulation of synthesis, 331
 tryptophane synthetase, 276-280
 tyrosinase, 31
- Enzyme adaptation, 321-322, *see also*
 Induced enzyme synthesis
- Enzyme-forming site, 321
- Episome, 144-146, 218-219, 243, 267, 344
- Epistasis, 345, 365
- Escherichia coli*, chromosome, 143-149
 control of DNA synthesis, 312
 controlling element, 334-338
 DNA replication, 188-191
 DNA synthesis timing, 185-186
 galactase fermenting mutants, 129-131, 146
 induced enzyme synthesis, 322-327
 lactose fermenting mutants, 46, 49, 136, 140, 145, 334-338
 mating, 134-136
 mating types, 135, 143-146
 mutation affecting histidine synthesis, 43, 49, 50
 mutation affecting methionine synthesis, 49-50
 mutation to streptomycin resistance, 40, 45
 nucleotides per map unit, 172
 permease, 331-334
 phenocopy, 380-381
 population dynamics, 382-384
 rate of enzyme synthesis, 336-338
 recombination, 134-149
 regulation of enzyme synthesis, 327-329
 repression of prophage, 344
 suppression, 298
 T phages, 22-24
 tryptophane synthetase, 276-280
- Euchromatin, 95
- Euglena*, 232, 253
- Evolution, 396
- Extra chromosomal, *see* Nonchromosomal
- F factor, 143-146, 219, 266-267
- F⁺, 135, 143-146

- F^- , 135, 137, 143–146
 F_1 generation, 86
 F_2 generation, 87
Fertility restorer, 258–259
Feulgen reaction, chromosomes, 178
 E. coli, 141
Fine structure, DNA in various organisms, 172
 phage, 163–167, 172
 Salmonella, 158–163
Fingerprinting, 280–282
First division segregation, 74–78, 254
Fitter types, 382–384
Fluctuation analysis, bacteria, 44–47, 51
 tissue culture, 376–377
Functional unit, *see* Unit of function
- Gal* mutants, 173
 linkage to lambda, 130–131, 146
 transduction, 129–131
Galactozymase, 321–322
Gametes, *Chlamydomonas*, 61
 Neurospora, 67–70
Gene, 2 action, 28–32
 controlling elements, 56–57, 334–338, 341–348
 definitions, 28, 32, 38–39, 150–152, 162, 168–173, 235–236
 distribution on chromosome, 303
 DNA nature, 20–22
 lethal, 57, 362
 mutable, 56–57, 341–346
 mutator, 56
 nonchromosomal, 242
 suppressor, 42, 171
Gene action, 30–31, 271ff
 DNA base ratios, 299–300
 irreparable mutants, 301–303
 rate of enzyme synthesis, 34
Gene conversion, 195–206
Gene dosage, 99
Genetic information, *see* Coding
Genetic maps, 64–66, 78–83
 E. coli, 137–148
 phage, 118–119
Genotype, 2, 86
Germ line, 357
Glucagon, 290
Glucose repression, 334
Glutamic dehydrogenase, 168
Golgi apparatus, 245, plate X, XIII, XVI
 Guanine, 5–13
 Guanosine, 5
 Guanylic acid, 5, 7
 Gynandromorph, 361
- Half chromatid, 183, 193–195
Haploid, 68–73
 DNA, 178–179
 sectors in *Aspergillus*, 364
Haploidization, 368–370
Heat, CO_2 sensitivity, 380
 Euglena chloroplasts, 253
 mutation, 229–230
 phenocopies, 380
Hemizygous, 137
Hemoglobin, 280
 amino acid sequence, 281
 recombination of polypeptides, 294–297
 tertiary structure, 287–290
Hereditary determinant, *see* Gene
Heredity, 1, 235
Heterochromatin, 95, 340–347
Heterocytosomes, 257
Heterogenote, 130–131, 145
Heterokaryon, 154–157, 291, 293, 301, 363–364, 371
Heterozygote, 85–89
 complementation, 291
 E. coli, 135–137, 142
 phage, 121
Hfr, 135, 137, 143–146
Histidine synthesis, regulation, 329–330
 transduction, 158–162
Histocompatibility, *see* Tissue incompatibility
Histone, 27, 179–180, 183–184
 in kinetoplasts, 246
Holmes rib grass virus, 24–27
Homozygote, 85–89
 E. coli, 142
Hot spot, 169
5-Hydroxymethyl cytosine, 8
Hyphae, 70
Hypomorph, 99
Hypotetraploid, 106
- i* mutants, 334–338
Inconstancy of affection, 87
Induced enzyme synthesis, 321ff
 kinetics, 326–327
 mechanism, 331

- Induced enzyme synthesis, phenocopy, 380-381
- Inducers of enzyme synthesis, 323-324
 action of, 331
 concentration, 380-381
 TMG, 324-325, 331-381
- Infectious heredity, 149, 262
F factor, 143-146
 nonchromosomal inheritance, 243
Paramecium, 264
Podospira, 260
 via phage, 125
- Insulin, 280
 amino acid sequence, 285
- Interference, chiasma, 79, 109
 chromatid, 79, 109-111
 negative, 80, 109, 118, 198-201
- Interphase, 70-71, plate V1
- Interrupted mating, 137-138
- Inversion, 101-104
- iojap*, 250-253, 258
- Irreparable function, 301-303
- Isoallele, 171
- K12, rH1 phage mutants and, 163-167, *see also E. coli*
- Kappa, 262-266
- Killer, 262-266
- Killing curves, 221-222
- Kinetoplast DNA, 178, 246
- Kinetosomes, 246
- Knobs, 94
- Lac* mutants, 46, 49, 136, 140, 145, 334-338
- Lambda (λ), 130-131
- Lampbrush chromosome, 94, 181-183
- Leukemia, 376-377
- Life cycles, 84
- Line, bacterial multiplication, 129
 transduction, 162
- Linkage, 73-78
Aspergillus, 154-158
Chlamydomonas, 64-66
 complementation, 191-193
 diploids, 89
E. coli, 137-148
 gene order and function, 162-163
 phage, 118-122, 163-167
Salmonella, 158-163
 somatic recombination, 365-370
 transformation, 19-22, 132-134
- Locus, 82
- Lozenge eye, 153
- Lysogenic conversion, 124-125
- Lysogeny, 122-125, 146
- Macronucleus, 262-263
- Maize, (*Zea mays*), chloroplast mutation, 249-253
 chromosome 9, 97
 controlling elements, 341-347
 cytological demonstration of crossing over, 107-109
 male sterility, 258-259
 meiosis, plates V, VI, and VII
 mutation, 218
 mutator gene *Dt*, 56-57
 nucleotides per map unit, 172
 paramutation, 261
- Male sterility, 258-259
 suppression, 298
- Mannitol fermenting pneumococcus, 19-20
- Mapping, 73-78
 by transduction, 158-163
 complementation, 191-193
 conventional and fine structure, 202-203
E. coli, 137-148
 fine structure, 173
 limitations, 78-82
 mitotic vs. meiotic, 368
 nonrandom distribution of genes, 303
 phage, 118-119, 163-167, 169
- Maternal inheritance, *see* Uniparental inheritance
- Mating type, *Chlamydomonas*, 60-61
E. coli, 135
Neurospora, 67, 70
- Meiosis, bacteria, 114, 142
 DNA content during, 185
 maize, plates V, VI and VII
Neurospora, 68-73
Paramecium, 262-263
- Mendel's first law, 62, 70
- Mendel's second law, 62, 73
- Metabolic block, 274-275
- Metaphase, 68-71, plates VI and VII
- 5-Methyl cytosine, 8
- Micronucleus, 262-263
- Microsomal particles, *see* Ribosomes
- Mitochondria, 246-249
Chlamydomonas, plate XVI
 liver, rat, plate I

- Mitochondria, mutants in *Neurospora*, 240, 254–257
mutants in yeast, 241, 254–257
pancreas, guinea pig, plate XIV
replications, 235
RNA in, 247–248
- Mitosis, 71–73
bacteria, 114, 142, 183
crossing over, 359–370
DNA content, 185
- Mitotic crossing over, *see* Somatic crossing over
- Monosomic, 106
- Mosaic, 374–375
- Mouse, nucleotides per map unit, 172
t-locus, 168
- Multiple allele, 171
- Mutable genes, 56–57, 341–346
- Mutable unit, *see* Unit of mutation
- Mutagens, acridine dyes, 232, 255–256
adenine, 212–213
alkylating agents, 217
2-amino purine, 169, 212
5-bromouracil, 169, 213–214
caffeine, 212
epoxides, 217
free radicals, 223, 229
heat, 216
nitrogen mustards, 217
nitrous acid, 36–38, 169
nonchromosomal factors, 232, 251–256
peroxide, 223, 229
proflavin, 216
sulphur mustards, 217
UV, 210, 227–229
X-ray, 103–104, 217–218, 219–225
- Mutant, auxotrophic, 274
constitutive, 334–338
cryptic, 332–333
definition, 35
increase in proportion, 208–209, 375
lethal, 221–222, 302
temperature sensitive, 277, 297, 301–302
- Mutation, and gene replication, 209–210
auxotrophy, 274
chemically induced, 169, 212–217, 231–232
chemostat, 207–209
definition, 35, 40–43
directed, 231–232
- Mutation, enzyme synthesis, 334–338
forward and back, 39–40, 43, 169–170
independence, 49–50
induced by nitrous acid, 36–38, 215–216
kappa, 264
lethal, 221–222, 302
nonchromosomal factors, 232, 251–257, 377–378
point mutations, 43, 217–219
position effect, 339–346
radiation induced, 219–231
randomness, 43–53
rates, 53–57, 208–209, 375–376
somatic cells, 372–378
temperature and, 210, 229–230
timing, 56–57, 342–346
unit, 40–43, 150–152
- Muton, 166–168, 170
- Mycelium, 70, 260
- Myoglobin, 289
- N¹⁵, 188–192
- Neurospora*, arginine synthesis, 29–31
complementation, 291–293
crossing over, 75–78
gene action, 273ff
life cycle, 67
meiosis, 68–69
mutation, 218
nucleotides per map unit, 172
poky mutant, 238–240, 254–257
RNA synthesis, 314
suppression, 297–298
tryptophane synthetase, 276–280
tyrosinase, 31
- Nitrogen base, 4
- Nitrous acid, 36–38, 169
- Nonchromosomal inheritance, 234ff
criteria, 241, 243
directed mutation, 232
F factor, 144
hypotheses, 242–244, 268–269
prophage, 148
- Nondisjunction, 91–93
Aspergillus, 369
E. coli, 135–137
- Non-Mendelian inheritance, *see* Nonchromosomal inheritance
- Nuclear envelope, 176, 245, plates IX, XV, XVI
- Nuclear transplantation, 351–352

- Nucleic acid, 4-12
 single-stranded, 313
 see also DNA and RNA
- Nucleolus, 176, 246, plate IX
 organizer, 94
 RNA, 27
 RNA synthesis, 316-317
- Nucleoprotein, 179-181
 bacteria, 183
 fibers, plate III
- Nucleoside, 4-5
- Nucleotide, 4-7
 per gene, 166-167
 sequence, 8
- Nucleus, 25, 68-73, 176
 bacterial, 141, 176, plate II
 Chlamydomonas, plate IX
 differentiation, 356-359
 incompatibility, 370
 kidney epithelium, rat, plate XIII
 liver, rat, plate I
 protein synthesis, 311
 transplantation, 351-353, 358
- o* mutants, 334-338
- One-gene-one-enzyme hypothesis, 30-31, 273-274
- Operator, *see* Controlling elements
- Origin of life, 392-396
- Ornithine *trans*carbamylase (OTC ase), 327-329
- Orthoselection, 384
- P₁ generation, 86
- P³², DNA replication, 185
 E. coli mapping, 141
 E. coli recombination, 142-143
 phage DNA, 22-24
 phage recombination, 122
 phage replication, 116-118
 prophage attachment, 146-147
 RNA synthesis, 314
 transformation, 16, 131-132
- Pachytene, 96, 100, 102, plate V
- Pairing, amphidiploids, 104-105
 DNA replication, 184-185
 inversion, 102
 oblique, 99
 pachytene, 96-97
 translocation, 100
- Pan-2* locus in *Neurospora*, complementation, 291-293
 recombination, 196-201
- Papain, 290
- Paramecium*, antigenic variation, 348-350
 life cycle, 262-263
 nonchromosomal inheritance, 264-266
- Paramutation, 261
- Parthenogenesis, 370
- Partial-replica hypothesis, *see* Copy choice
- Penicillin resistance, 17-22
- Penicillinase, 322-323, 326, 334-335
- Peptide, 280
- Periodic selection, *see* Orthoselection
- Permease, 172, 331-334
 genetic control, 334-336
- Petite mutants, 240-241, 254-257
- Phage, biochemistry of infection, 114-118
 DNA, 22-24
 fine structure of genes, 163-167
 genetics, 118-131
 heterozygotes, 121
 immunity, 124-125
 lambda (λ), 130-131, 146-148
 mutation, 45
 nucleotides per map unit, 172
 r mutant mapping, 81-83
 temperate, 122-125
 virulence, 124
- Phase variation, 347-348
- Phenocopy, 4, 380-382
- Phenotype, 2, 42, 86, 271-273
- Phi (ϕ)X174, single-stranded DNA, 312-313
- Photoreactivation, 225-226
- Photosynthesis, 248-249
- Plant pigments, 271-273
- Plaque, 82, 114
- Plasma membrane, 245, plates I, II, IX, XII, XIII, XV, XVI
- Plastid, *see* Chloroplast
- Pleiotropism, 272
- Pneumococcus (*Diplococcus pneumoniae*),
 mutation with nitrous acid, 37
 polysaccharide capsule, 12, 20, 132-134
 transformation, 12-22, 131-134
- Podospira*, 260-261
- Poisson distribution, 48-51, 53, 79
- Poky* mutant, 238-240, 254-257
- Polka-dot bodies, (multiviscular bodies),
 plate XII

- Pollen, 85
- Polynucleotide, 4–12
 complementarity, 10
- Polynucleotide phosphorylase, 312
- Polypeptide, 284, 294–297
- Polyploidy, 104–106, 370
- Polytene chromosome, 94, 106
- Population dynamics, 382–385
- Position effect, 95, 153, 163, 218, 338–347
 Oenothera, 341
- Position pseudoalleles, 152–158, 340
- Primary structure, 284–291
- Prophage, 122–125
 chromosomal location, 146–148
 defective, 124–125, 130–131
 integration, 266–267
 noninducible, 146–147
 transduction of, 127–128
- Prophase, 68–71, plate V and VII
- Protamine, 179
- Protein, as genetic material, 27, 28
 induced synthesis, 32ff
 phage, 22–24
 regulation of synthesis, 331
 structure, 283–291
 synthesis, 308–311, 320–321
- Prototroph, 50, 138
- Provirus, 379–380
- Pseudoallelism, 154–158, 163, 166, 172, 196
- Pseudomonas* recombination, 148
- Pseudouracil, 309
- Puffs, 318–320, 358
- Purine, 5
- Pyrimidine, 5
- Quaternary structure, 284–285
- r* mutants in phage, 81–83, 119, 163–167, 170
- Radioautography, 116–118, 185
 in DNA synthesis, 192–195
 in RNA synthesis, 314–315, 318–321
- Radiomimetic agents, 217
- Rana pipiens*, 351–353
- Rat lethal mutation, 272–273
- Recessive, 86–89
 versus deficiency, 98–99
- Reciprocal crossing, maize, 249–250
 Neurospora, 239
 Podospora, 260–261
- Recombination, 64, 73–78, 177
 E. coli, 134–149, 336–338
 models of, 202–206
 Neurospora, 72–78, 196–202
 nonreciprocal, 198
 phage, 118–122
 pneumococcus, 132–134
 Salmonella, 158–162
 unit of, 150–152
- Recon, 166–168
- Regulation, arginine synthesis, 327–329
 by glucose, 334
 enzyme synthesis, 331, 336–338
 histidine synthesis, 329–330
- Reparable function, 301
- Repeat, *see* Duplication
- Replica plating, 52–53
 in bacterial mating, 143
- Replication, 173
 as life, 395–396
 asynchronous, 195
 in heredity, 235–236
 of cell structures, 244
 of DNA, 177, 184–195
- Repression, antibody formation, 384
 arginine synthesis, 327–329
 chromosome rearrangement, 340–347
 enzyme synthesis, 331, 336–338
 gal locus, 344
 glucose, 334
 histidine synthesis, 329–330
 mechanism, 331, 338, 350
 Paramecium, 348–350
 prophage, 146, 344
 Salmonella, 347–348
- Ribonuclease, 285–287
- Ribonucleic acid, *see* RNA
- Ribose, 5, 7
- RNA, as genetic material, 24–27, 236
 cell localization, 244–246
 chromosomes, 180
 composition of, 4–12
 DNA synthesis, 229
 encephalitis virus, 25
 heterogeneity of, 313
 Holmes rib grass virus, 24–27
 mitochondria, 247–248
 phage infection, 114
 polio virus, 25
 reaction with nitrous acid, 36–39
 soluble (transfer), 308–311

- RNA, synthesis, 312-321
 TMV, 24-27
- Ribosomes (microsomal particles), 245-246
Chlamydomonas, 245, plate XVI
E. coli, 245-246, plate II, IV
 kidney, rat, plate XII
 liver, rat, plate I
 pancreas, guinea pig, plate XI
 protein synthesis, 308-311
 RNA in, 27
- S³⁵, phage protein, 22-24
 protein synthesis, 309, 323
 TMG³⁵ and permease, 332-333
- S-type position effect, 340
- Salivary chromosome, *Chironomus*, 320-321
Drosophila, 80-81, 94, 99, 316, plate VIII
 nucleic acid synthesis, 318-321
 puff, 318-320
Rhynchosciara, 316, 318-320
Sciara, 320-321
- Salmonella*, genetic fine structure, 158-163
 induced mutation, 211-212
 phase variation, 347-348, 350
 recombination, 148
 regulation of enzyme synthesis, 329-330
 transduction, 125-130
- Second division segregation, 74-78
- Secondary structure, 284
- Selection, antibody formation, 384
 bacteria, 43-53, 382-384
 fungal heterokaryons, 370
 organic molecules, 394
 tumor cells, 373
- Senescence, 260, 380
- Sex, 2, 113-114
- Sex determination, 92, 370
- Sex-linked inheritance, 91-93
- Sex ratio, 264-268
- Sex-reversal, 358
- Sexual spores, 70
- Shigella dysenteriae* recombination, 148
- Sickle cell anemia, 280
 trait, 280
- Sigma (σ), 380
- Sister-strand exchange, 109-111, 191-195,
 202-206
- Soma, 357, 371
- Somatic crossing over, *Aspergillus*, 362-370
Drosophila, 359-362
 reciprocal products, 365
- Somatic reduction, 367-370
- Somatic segregation, 250-253, 258
Aspergillus, 362-370
Drosophila, 359-362
- Sordaria*, 200-201
- Spindle, 68-71, 177
- Status albobaculatus*, 249-253
- Steady state as heredity, 243
- Streptomyces*, recombination, 148
- Streptomycin, effect on chloroplasts, 232, 253
- Streptomycin dependence, 40
- Streptomycin resistance, *Chlamydomonas*,
 80, 236-238, 259
E. coli, 40, 47, 51-53
 pneumococcus, 17-22
 transduction, 125-128
- Suppressor gene, 42, 171
 CRM in suppressed mutants, 297-298
 definition, 297
- Symbionts, 378
- Syngamy, 61
- t* locus in the mouse, 168
- Target theory, 219-222
- td* mutants, 276-280, 297-298
- Telophase, 68-73, plate VI
- Template, 121, 173, 186-188, 242
- Tertiary structure, 284
 and amino acid composition, 289-291
 hemoglobin, 287-290
 myoglobin, 289
 ribonuclease, 286-287
- Test cross, 89
- Tetrads, 80, 197
- Tetraploid, 106
Aspergillus, 363
- Thiomethyl galactoside (TMG), 324-325,
 327, 331-333
- Three-point cross, in phage, 119
 in *Salmonella* transduction, 158
- Thymidine, and DNA replication, 185, 192
 localization on chromosomes, 312, 318-320
- Thymine, 5-12
- Thymineless death, 312
- Tissue culture, 106
 asynchronous chromosome duplication,
 195
 chromosome number, 376
 DNA replication, 189
 mutation, 375-378
 RNA synthesis, 315-316

- Tissue culture, virus infection, 378–379
Tissue incompatibility, 372–374
TMV (tobacco mosaic virus), 24–27
 mutation with nitrous acid, 37–38
trans-arrangement, 153–158
 in phage, 165–166
Transduction, 125–131
 abortive, 129–130
 and enzyme synthesis, 336–338
 by *F* factor, 144–146
 phase variation, 347–348
Transformation, 4
 Hemophilus, 133–134
 pneumococcus, 12–22, 131–133
Transforming principle, 15
Translocation, 100–101
Transposition of controlling elements, 342–347
Trisomic, 93, 106
Trypanosoma cruzi, 246
Tryptophane synthesis transduction, 158–162, 278–279
Tryptophane synthetase, 276–280, 297–298
Tumor, 372–374, 376–377, 379
Twin spot, 359
Tyrosinase, 31, 274, 277

Ultraviolet light, absorption by nucleic acids, 27
 action spectrum, 224
 and recombination in phages, 122
 bacterial recombination, 142
 diploidization, 363
 DNA and mutation, 227–229
 Euglena chloroplasts, 253
 induction of mutation, 223–225
 nuclear number in *E. coli*, 141
 origin of life, 393
 photoreactivation, 225–226
 somatic recombination, 370
 temperate phages, 124, 344
Unequal crossing over, 339
Uniparental inheritance, 238–241
Unit of function, 32, 39, 150–152, 158–163, 167–173
Unit of mutation, 40–43, 150–152
Unit of transmission, 150–152

Unsolved problems, *see* Laboratory
Uracil, 5
Uridine, 5
 tritiated and RNA synthesis, 314
Uridylic acid, 5–7

V-type position effect, 340–341
Variegated pericarp, 344
Variegation, *see* V-type position effect
Vegetative phage, 115, 123
Viruses, CO₂ sensitivity, 379–380
 encephalitis, 25, 378
 Holmes rib grass virus, 24–27
 influenza, 378
 mammary tumor, 379
 polio, 25, 378
 provirus, 379
 Shope papilloma, 379
 somatic variation, 378–380
 TMV, 24–27
 see also Phage

Waring Blendor, bacterial recombination, 136–137
 phage experiment, 23–24
 zygotic induction, 146
Wheat (*Triticum*), 105
Wild type, definition of, 35–36

X chromosome, 92
X-ray crystallography, 287–289
X-rays, antigenic variation, 373
 chromosome breakage, 94–98
 induction of mutations, 103–104, 217–218, 219–225, 302
 virus-induced tumor, 379

Y chromosome, 92, 95
y mutants, 334–338
Yeast, (*Saccharomyces cerevisiae*), 83, 240–241, 254–257
 galactozymase, 321–322

z mutants, 334–338
Zygote, *Chlamydomonas*, 61
 Neurospora, 67–70
Zygotic induction, 146

